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1314671

THE UNITED STATES OF AMERICA

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United States Patent and Trademark Office

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APPLICATION NUMBER: *60/557,740*
FILING DATE: *March 29, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/10233*

Certified by

Jon W. Dudas

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. ER 388500347 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Robin L.	Polt	Tucson, Arizona

Additional inventors are being named on the 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Amphipathic Helical Glycopeptide Address Sequences for Enhanced Blood-Brain Barrier Transport of Neuroactive Peptides

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ Customer Number: 021368

OR

☐ Firm or Individual Name	David G. Perry				
Address	Office of Technology Transfer; The University of Arizona				
Address	888 N. Euclid Ave., Rm. 204; P.O. Box 210158				
City	Tucson	State	AZ	Zip	85721
Country	USA	Telephone	520-621-5000	Fax	520-626-4600

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 44 ☐ CD(s), Number _____

☐ Drawing(s) *Number of Sheets* _____ ☐ Other (specify) _____

☐ Application Date Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees.

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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☑ Yes, the name of the U.S. Government agency and the Government contract number are: Navy/ONR; N00014-02-1-0471

Respectfully submitted

SIGNATURE David G. Perry

TYPED or PRINTED NAME David G. Perry

TELEPHONE 520-621-5000

Date 3/29/2004

REGISTRATION NO. 34,405 *(if appropriate)*

Docket Number: UA 04-065

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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Docket Number UA 04-065

INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Dhanasekaran	Muthu	Tucson, Arizona

Number 2 of 2

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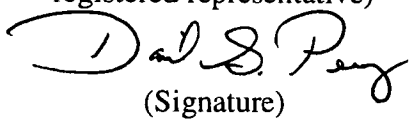
David G. Perry
(Name of Applicant, assignee or
registered representative)



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U.S. Provisional Patent Application

TITLE: Amphipathic Helical Glycopeptide Address Sequences for Enhanced Blood-Brain Barrier Transport of Neuroactice Peptides

INVENTORS: Robin L. Polt & Dhanasekaran Muthu

FILED: March 29, 2004

Glycopeptide Analgesics: Conformational and Pharmacological Characterization of O-Linked Glycosyl-Enkephalins and Glycosyl-Endorphins

Robin Polt, Dhanasekaran Muthu, Edward J. Bilsky, Henry I. Yamamura, Frank Porreca, Larissa Yeomans, Charles M. Keyari, Richard D. Egleton

Endogenous Opioid Peptides

Tyr-Gly-Gly-Phe-Met

Tyr-Gly-Gly-Phe-Leu

Enkephalins

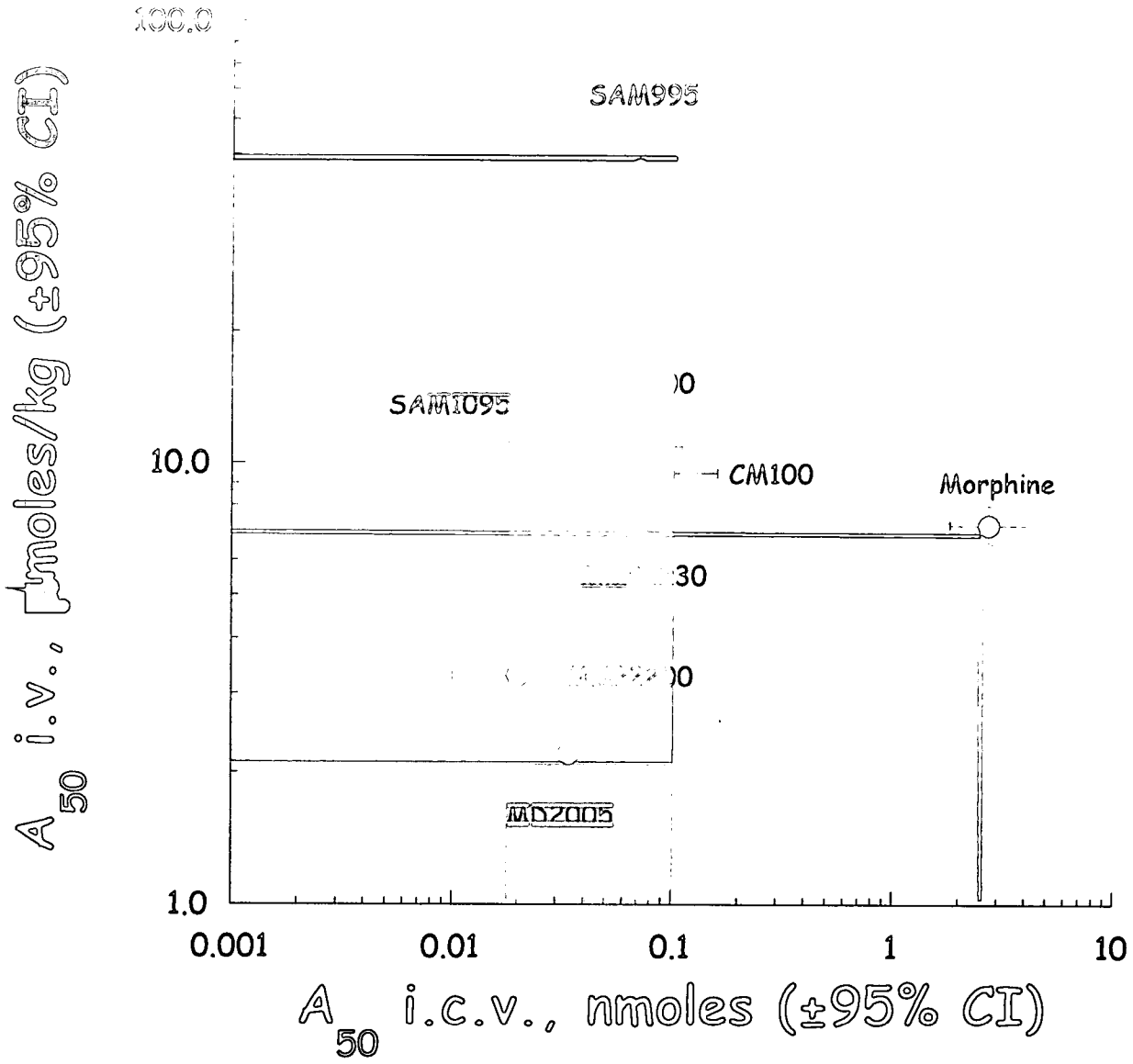
Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu^{31}

beta-Endorphin

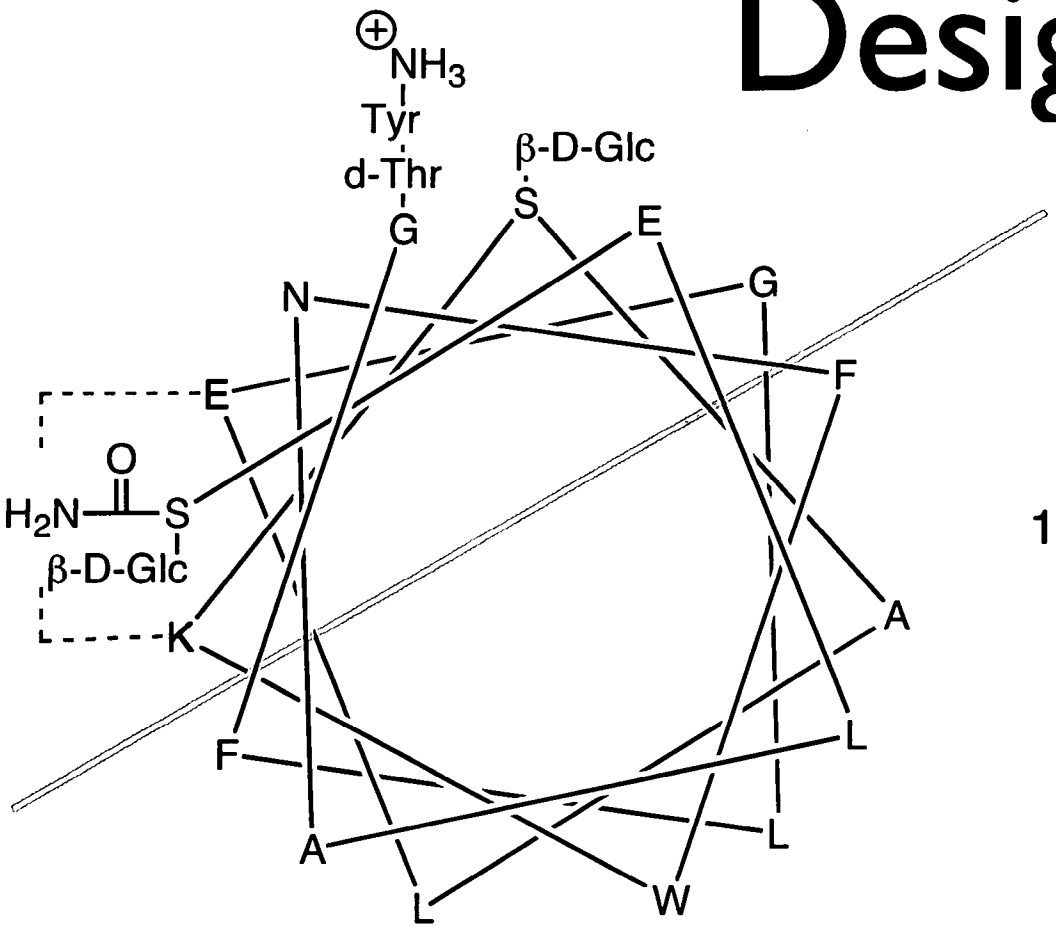
Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asn-Asn-Gln^{17}

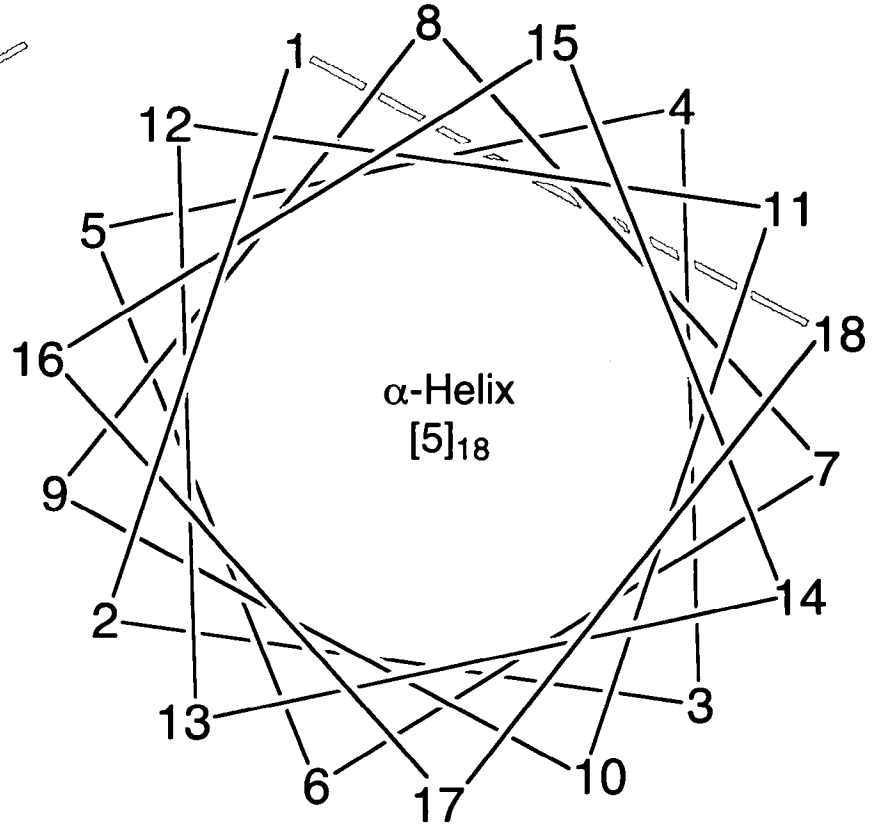
Dynorphin-A

i.v. vs s.c. Injection

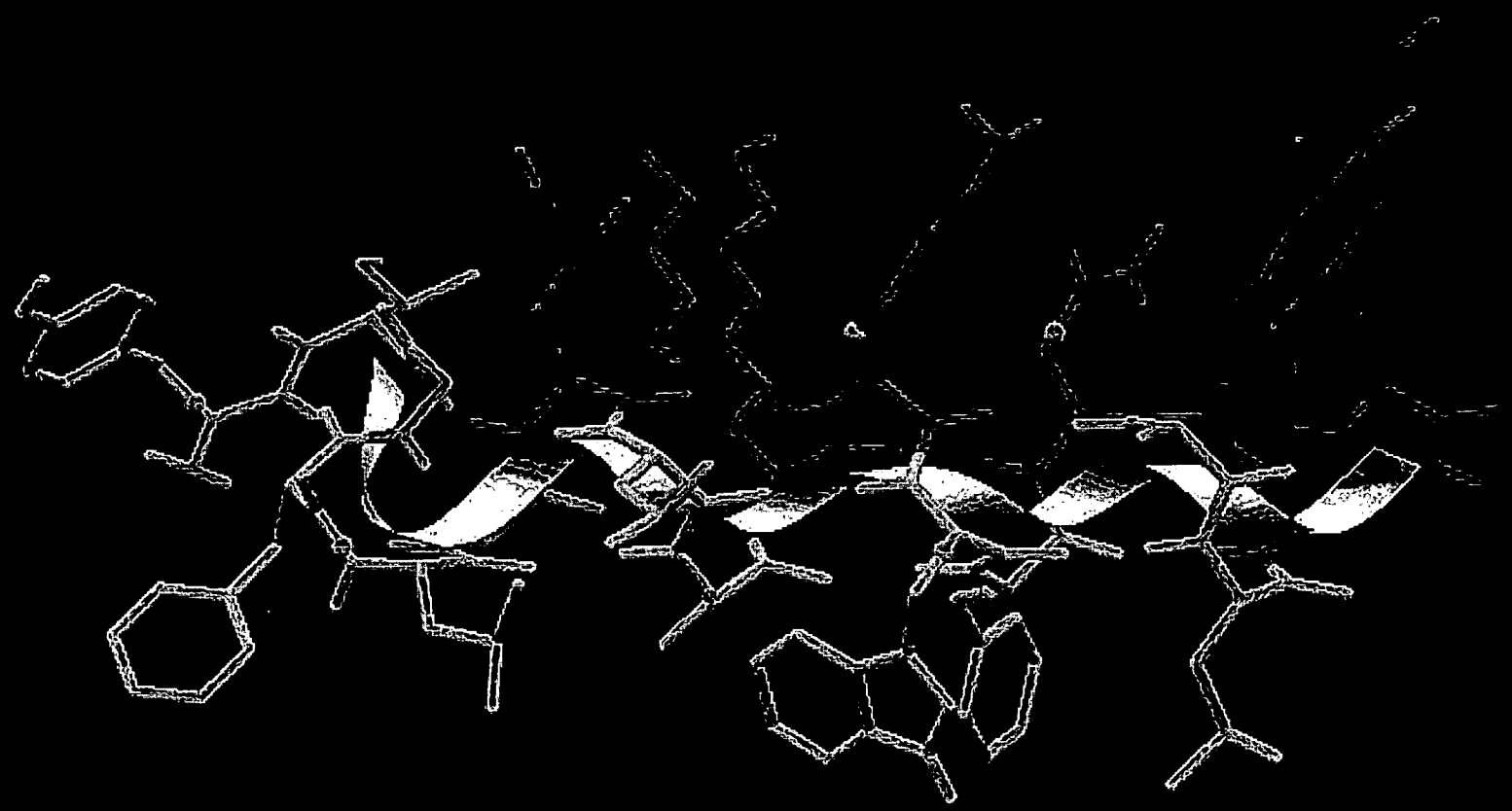


1st Generation Helix Design





YtGFLGELAS*-KWFNA-LES*-$CONH_2$

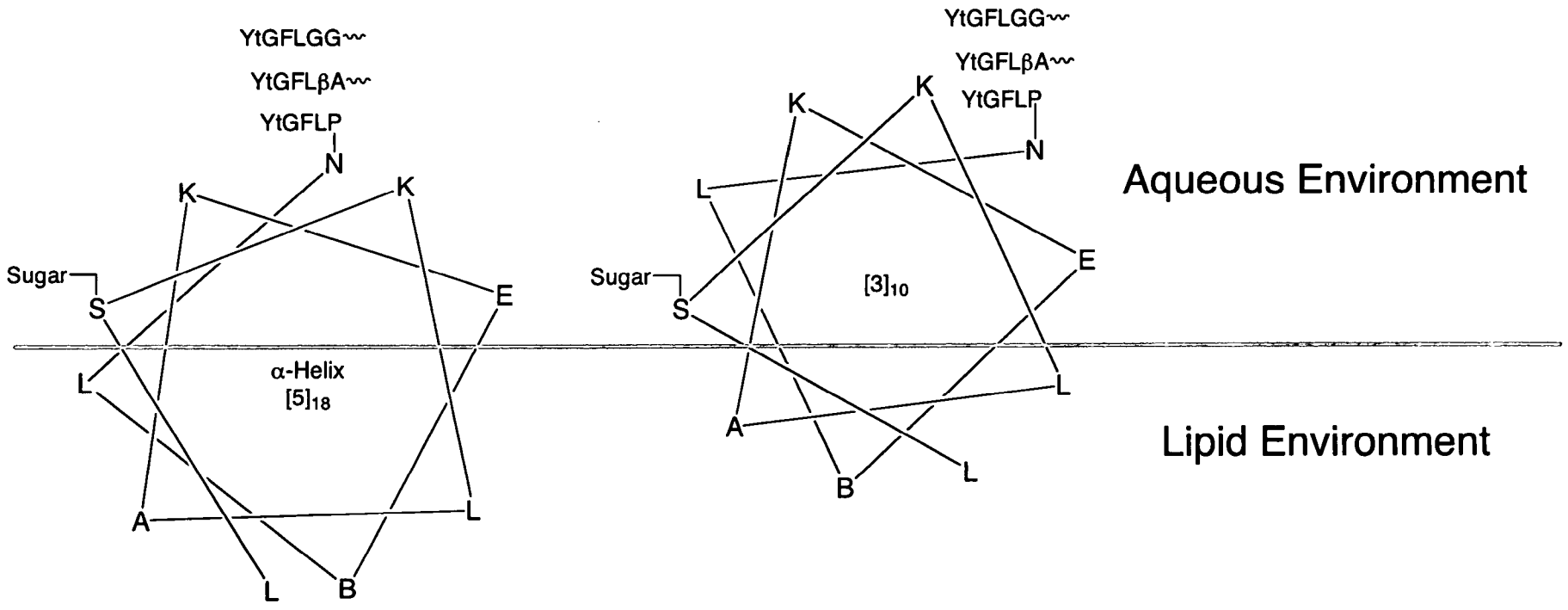


YtGFLGELAS*KWFNALES*-$CONH_2$

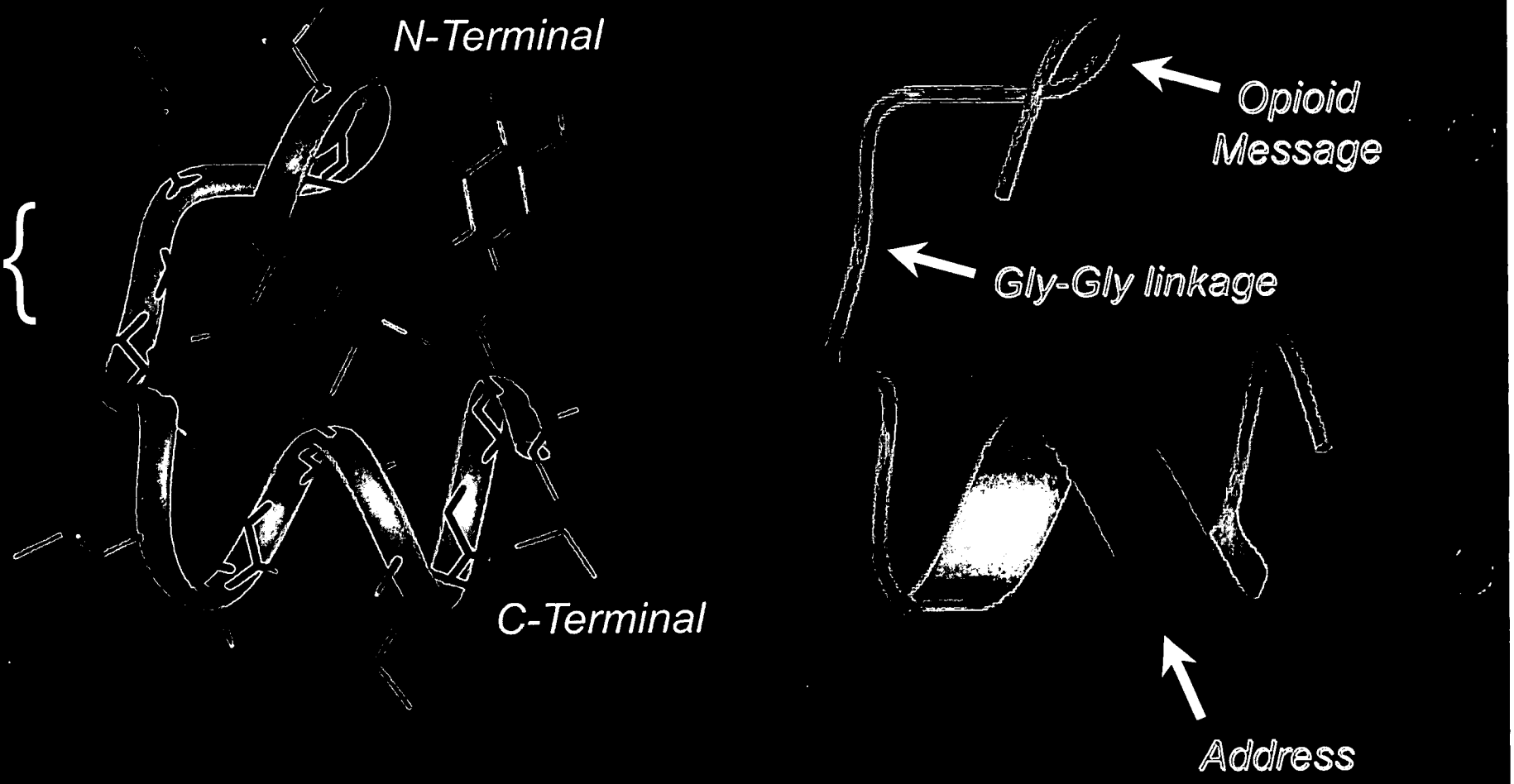
1st & 2nd Generation Helical Opioids

Peptide Sequence	*delta* IC_{50} nM	*mu* IC_{50} nM
YtGFLGELAS*KWFNALE-$CONH_2$	insoluble in H_2O	
YtGFLGELAS*KWFNALES*-$CONH_2$	9.5	144
YtGFLGELAS*KWFNALES*F-$CONH_2$	insoluble in H_2O	
YtGFLGELAS*KWFNALES*FW-$CONH_2$	insoluble in H_2O	
YtGFLGALKS*FAES*LS*N-$CONH_2$	—	—
YtGFLGLLKS*FAES*WS*NF-$CONH_2$	11.9	154
YtGFLGKS*FAELWS*NFLS*-$CONH_2$	25.6	38.2
YtGFLGLLKS*FWES*WS*NF-$CONH_2$	—	—

3rd Generation Helix Design

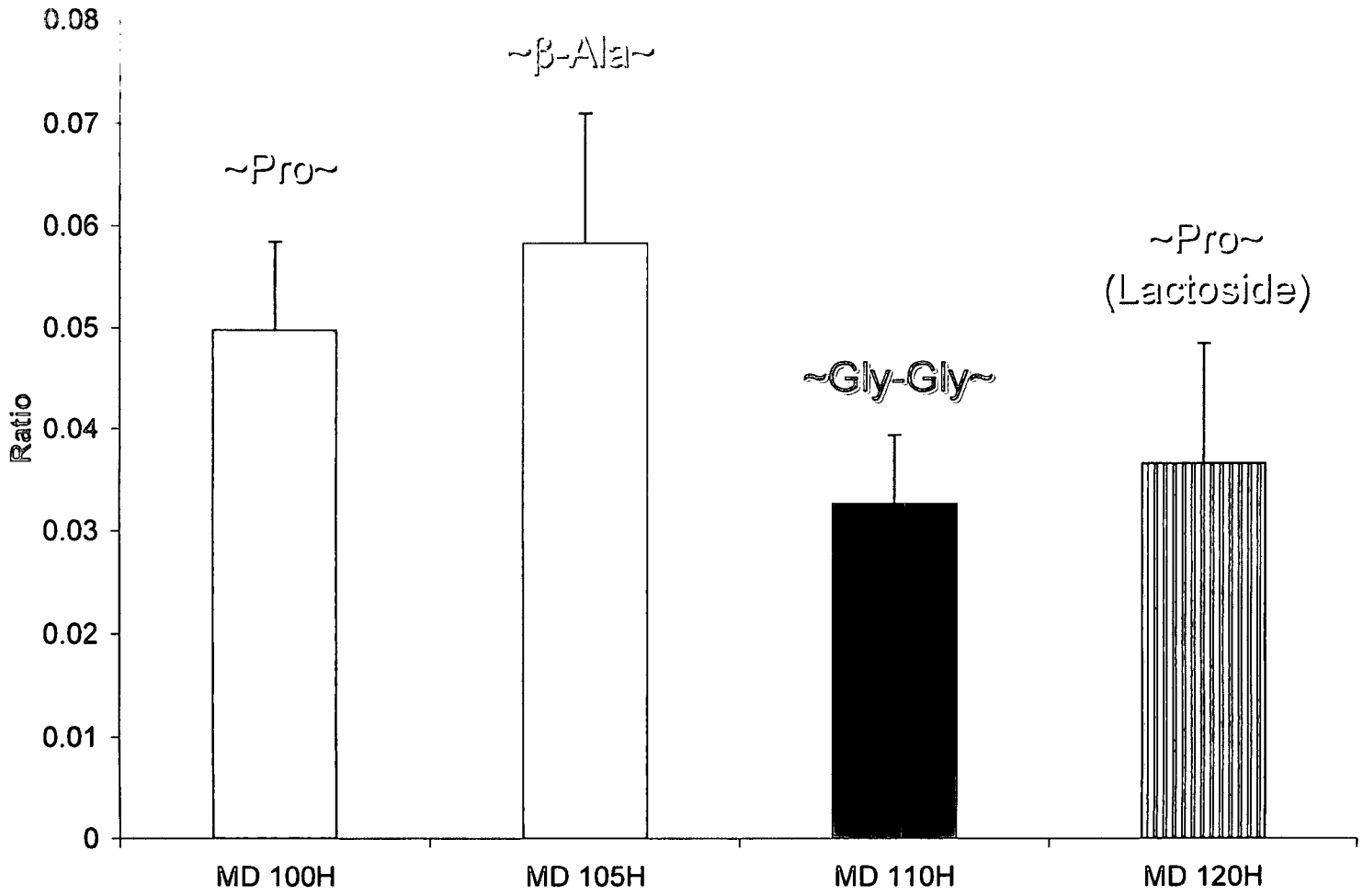


3rd Generation Helices

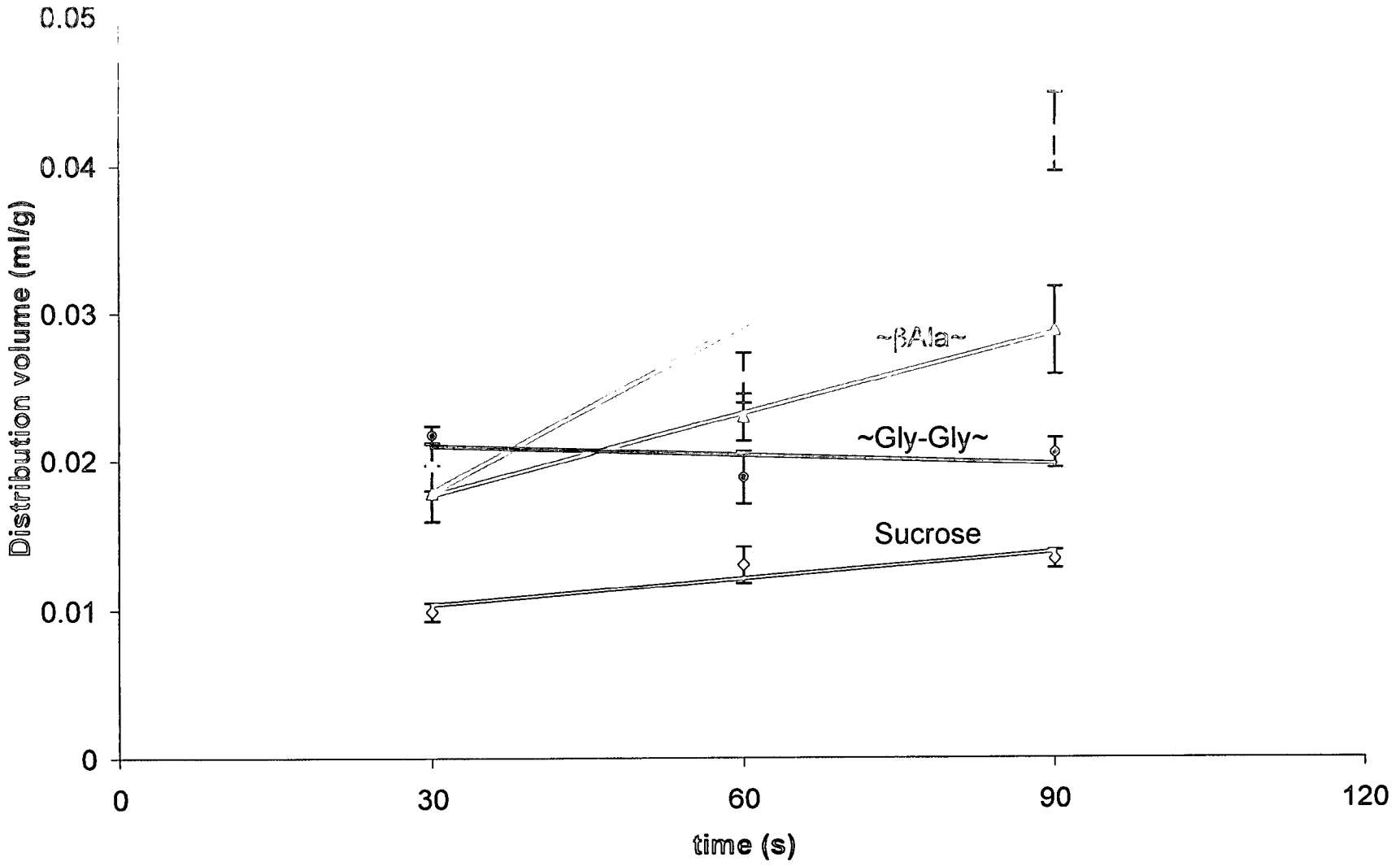


Dhana Muthu *(unpublished results)*

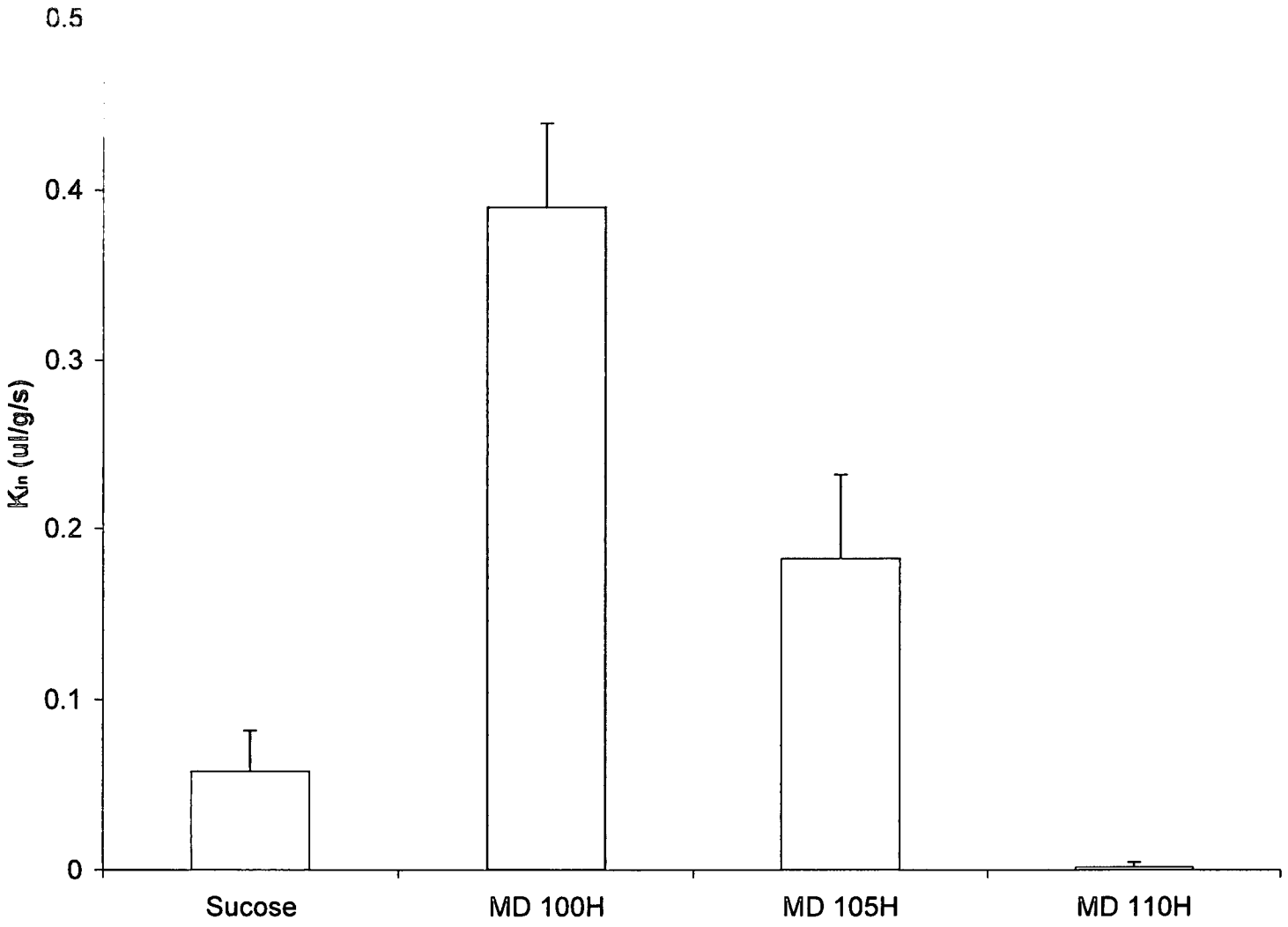
Octanol:Saline Distribution Studies



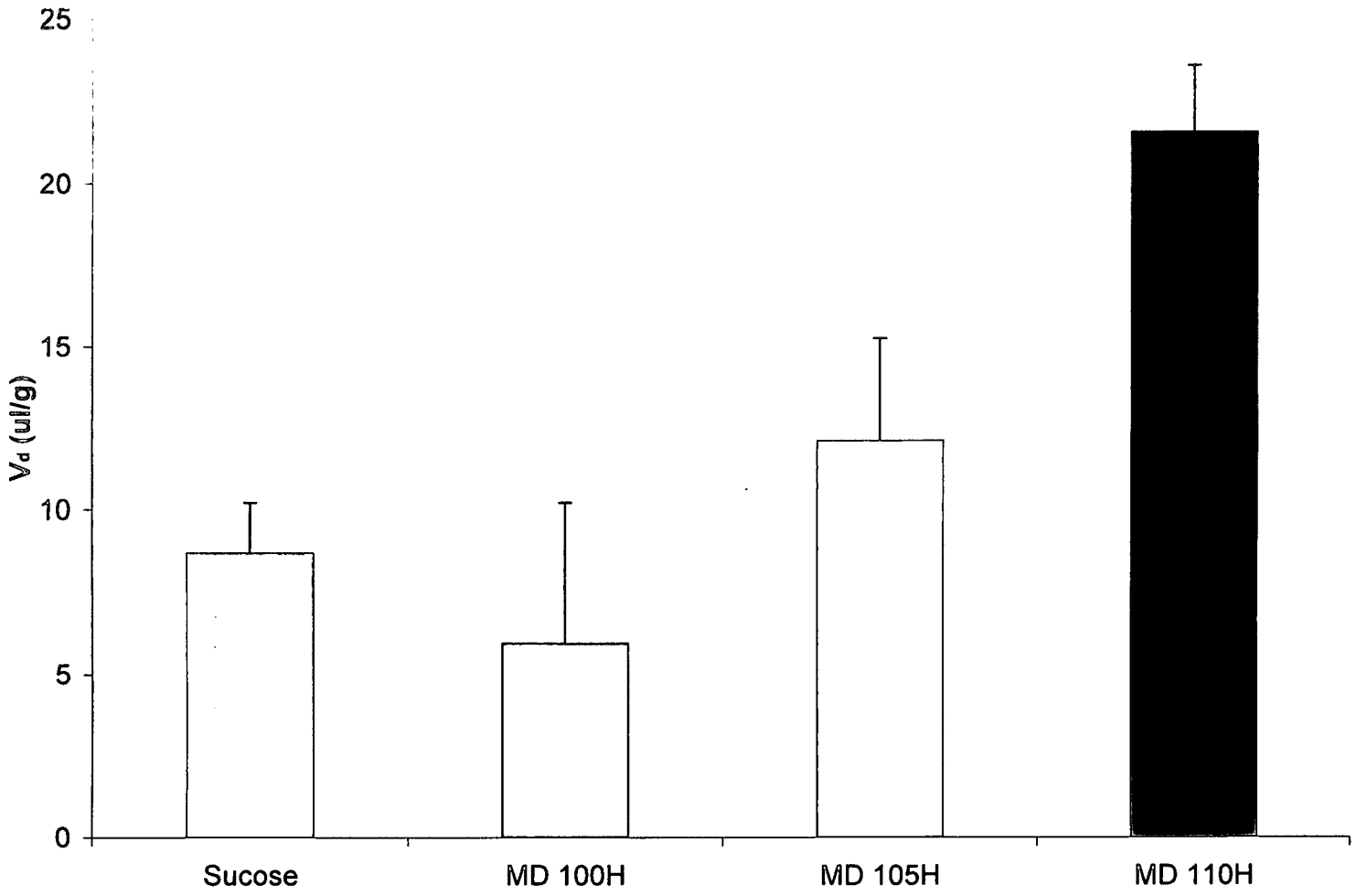
Mouse *in situ* Perfusion Studies



K_{in} Values from *in situ* Perfusion Studies



Initial Volume of Distribution (V_d) from *in situ* Perfusion Studies



Functional Bio-Assays

H_2N-Y-t-G-F-L-Linker-N-B-E-K-A-L-K-Ser(Glc)-L-NH_2

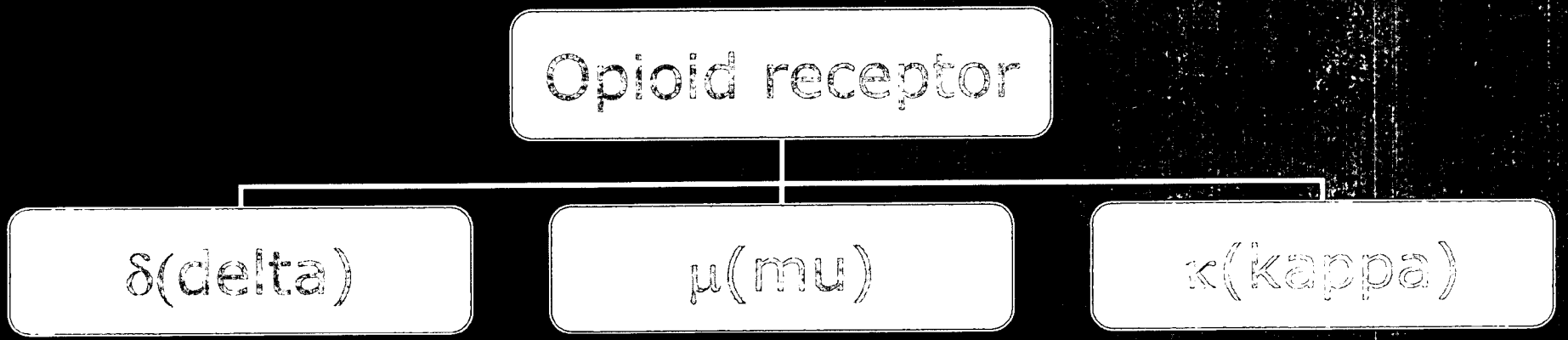
Linker	MVD (IC_{50})	GPI (IC_{50})	Ratio (delta/mu)
Pro	34.5 nM	63.1 nM	1.8
beta-Ala	23.0 nM	354 nM	15
Gly-Gly	18.8 nM	196 nM	10
Morphine	258 nM	54.7 nM	0.21

Peg Davis, U. of A. Pharmacology (*unpublished*)

Glycopeptide analgesics: Conformational and pharmacological characterization of O-linked glycosyl-enkephalins and glycosyl-endorphins

Dhanasekaran Muthu Ph.D.
Prof. Robin Polt's Laboratory
Department of Chemistry
The University of Arizona
Tucson 85721

Opioid receptor and their agonist effects



- Analgesia

- Analgesia
- Respiratory depression
- Miosis
- Reduced
  -gastrointestinal motility
- Nausea
- Vomiting
- Euphoria

- Analgesia
- Respiratory depression
- Miosis
- Dysphoria

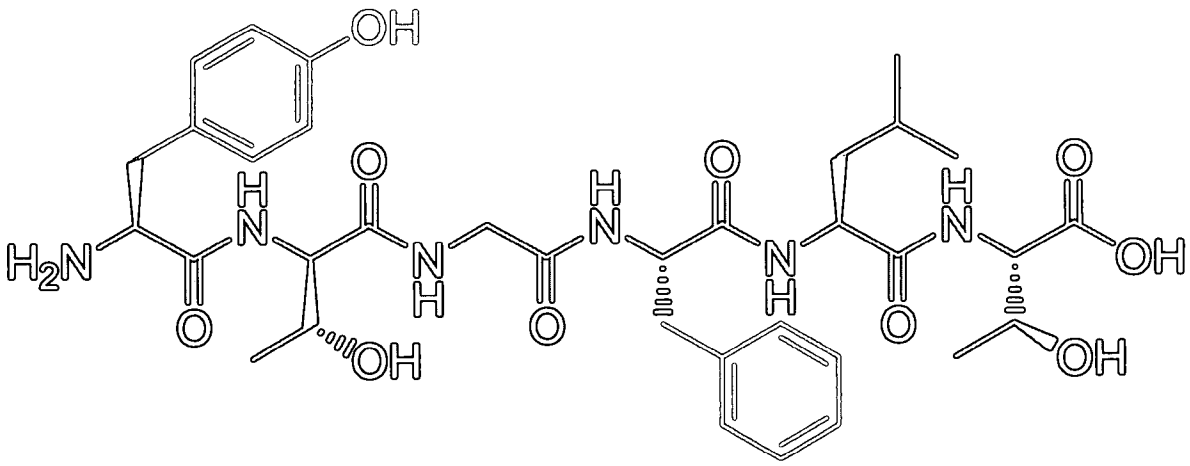
M.J. Brownstein, *Proc.Natl.Acad.Sci. USA* **90**:5391-5393(1993)

Naturally occurring opioid peptides

Peptide	*Sequence*	*Receptor Subtype*
Met-Enkephalin	YGGFM	μ/δ
Leu-Enkephalin	YGGFL	δ/μ
Dynorphin A	YGGFLRRIRPKLKWNNQ	κ(μ)
Dynorphin B	YGGFLRRQFKVVT	κ(μ,δ)
α-Neoendorphin	YGGFLRKY	κ(μ,δ)
β-Neoendorphin	YGGFLRKYP	κ(μ,δ)
β_h-Endorphin	YGGFMTSEKSQTPLVTLFKNAIIKNAYKKGE	μ/δ
Peptide E	YGGFMRRVGRPEWWMDYQKRYGGFL	μ/κ

Linear Leu-enkephalin analog

Tyr-DThr-Gly-Phe-Leu-Thr-OH (DTLET)

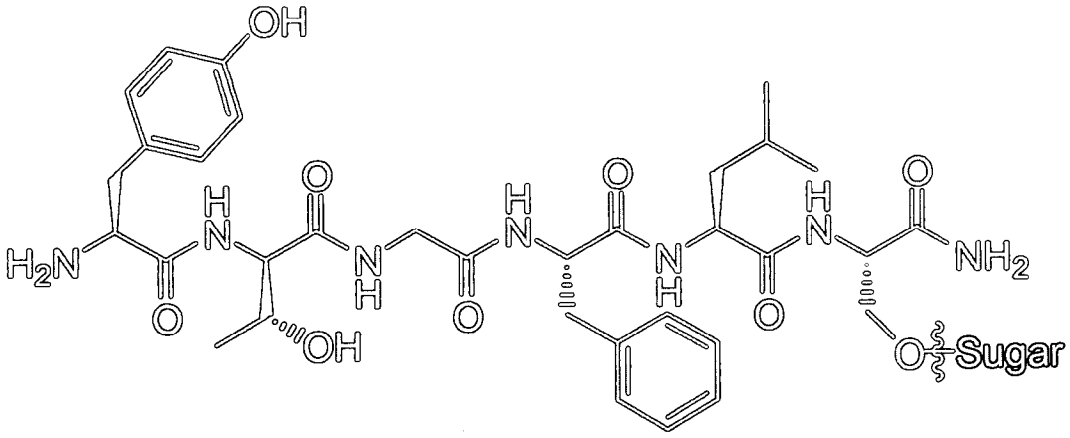


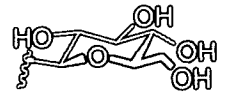
δ selective agonist

G.Gacel et. al. J. Med. Chem., 31:1891-1897(1988)

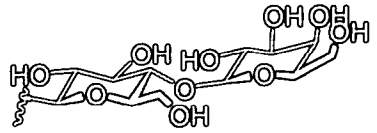
Glycosylated enkephalin analogue

TYR-(D)THR-GLY-PHE-LEU-SER*-NH_2

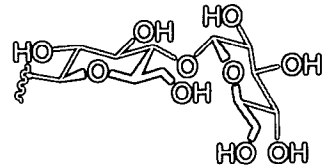




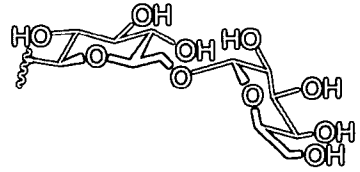
β-D-Glucose



B-Lactose



β-Maltose



β-Melibiose

R.Polt et. al. *Proc.Natl.Acad.Sci. USA*. 91:7114-7118(1994)
E.J.Bilsky et. al. *J.Med.Chem.* 43:2586-2590(2000)
S.A.Mitchell et al. *J.Org.Chem.* 66:2327-2342(2001)

Advantages of glycosylated opioid peptide analogs

- Highly water soluble
- Increased serum stability
- Blood brain-barrier is not a problem
- Simple metabolites (amino acid and sugar)
- No side-effects shown on mice, yet

R.Polt et. al. *Proc.Natl.Acad.Sci. USA*. 91:7114-7118(1994)
E.J.Bilsky et. al. *J.Med.Chem.* 43:2586-2590(2000)
R.D.Egleton et. al. *J.Pharm.Expt.Ther.* 299:967-972(2001)
R.D.Egleton et. al. *Brain research* 881:37-46(2000)

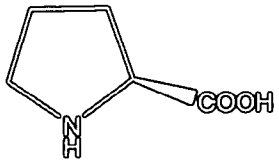
Design of helical endorphin/dynorphin analogs

Message segment

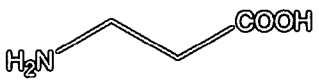
Address segment

H_2N-Y-(D)T-G-F-L-Linker-N-B-L-E-K-A-L-K-S*-L-NH_2

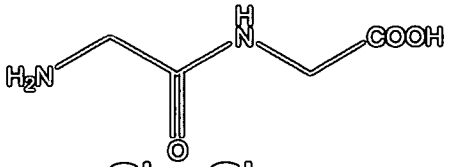
Designed to be amphipathic helical



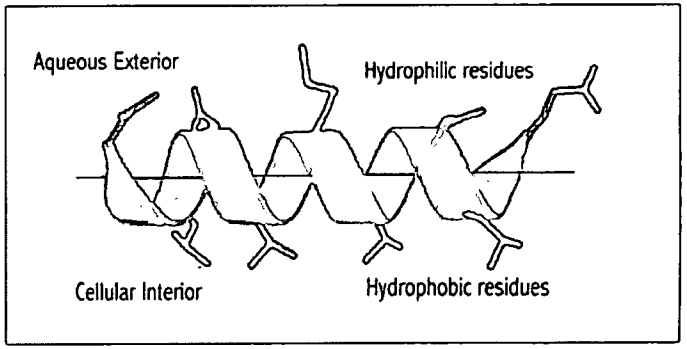
Proline



β-Alanine

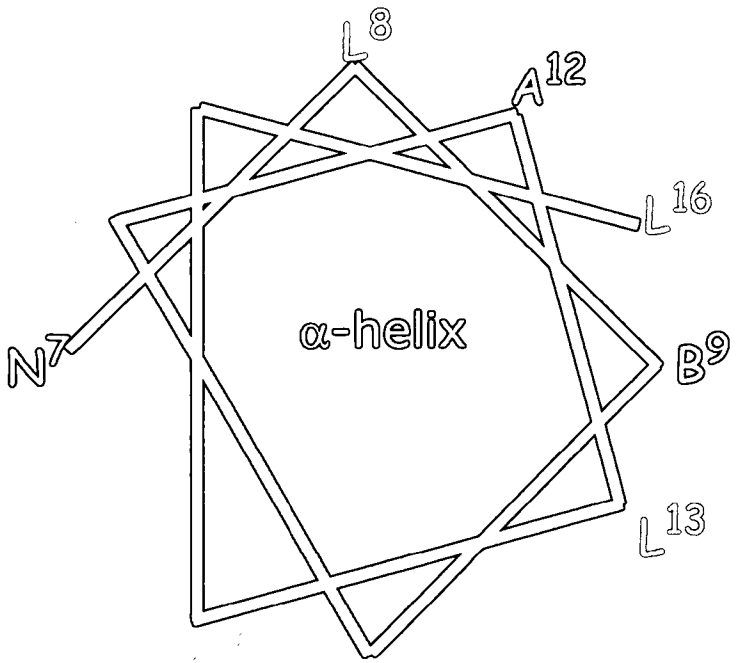


Gly-Gly



Design of amphipatic helical address segment

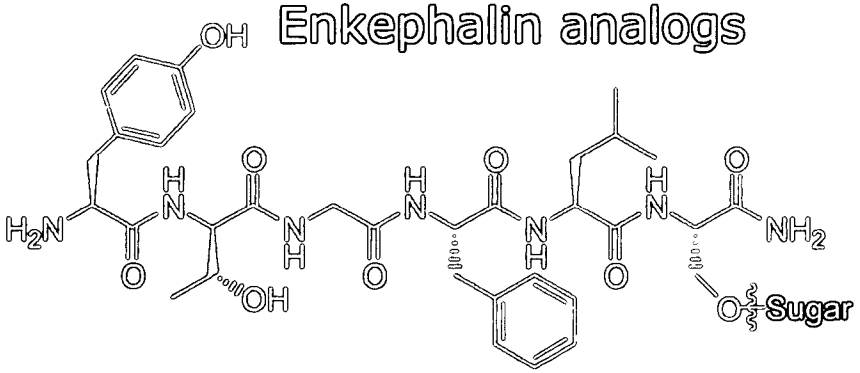
N^{7}-L^{8}-B^{9}-E^{10}-K^{11}-A^{12}-L^{13}-K^{14}-$\underline{S(Sugar)^{15}}$-$L^{16}$



- Purely based on amino acid secondary structure propensity and hydrophobic character
- Asn^{7} as helix cap
- Salt bridge between Glu^{10} and Lys^{14} to improve solubility and helix stability
- Unnatural amino acid to Aib^{9} promote helix formation
- Amino acid heterogenity maintained for NMR characterization

Pharmacology: in vitro binding

Enkephalin analogs



Sugar	MVD (δ) IC_{50} nm	GPI (μ) IC_{50} nm	μ / δ selectivity
No sugar	2.723	25.04	9.1
Glucose	1.56	33.83	21.6
Lactose	5.727	34.75	6.1
Melibiose	6.062	63.14	10.4
Morphine	258	54.7	0.212

Peg Davis, Dept of Pharmacology, University of Arizona

Pharmacology: in vitro binding

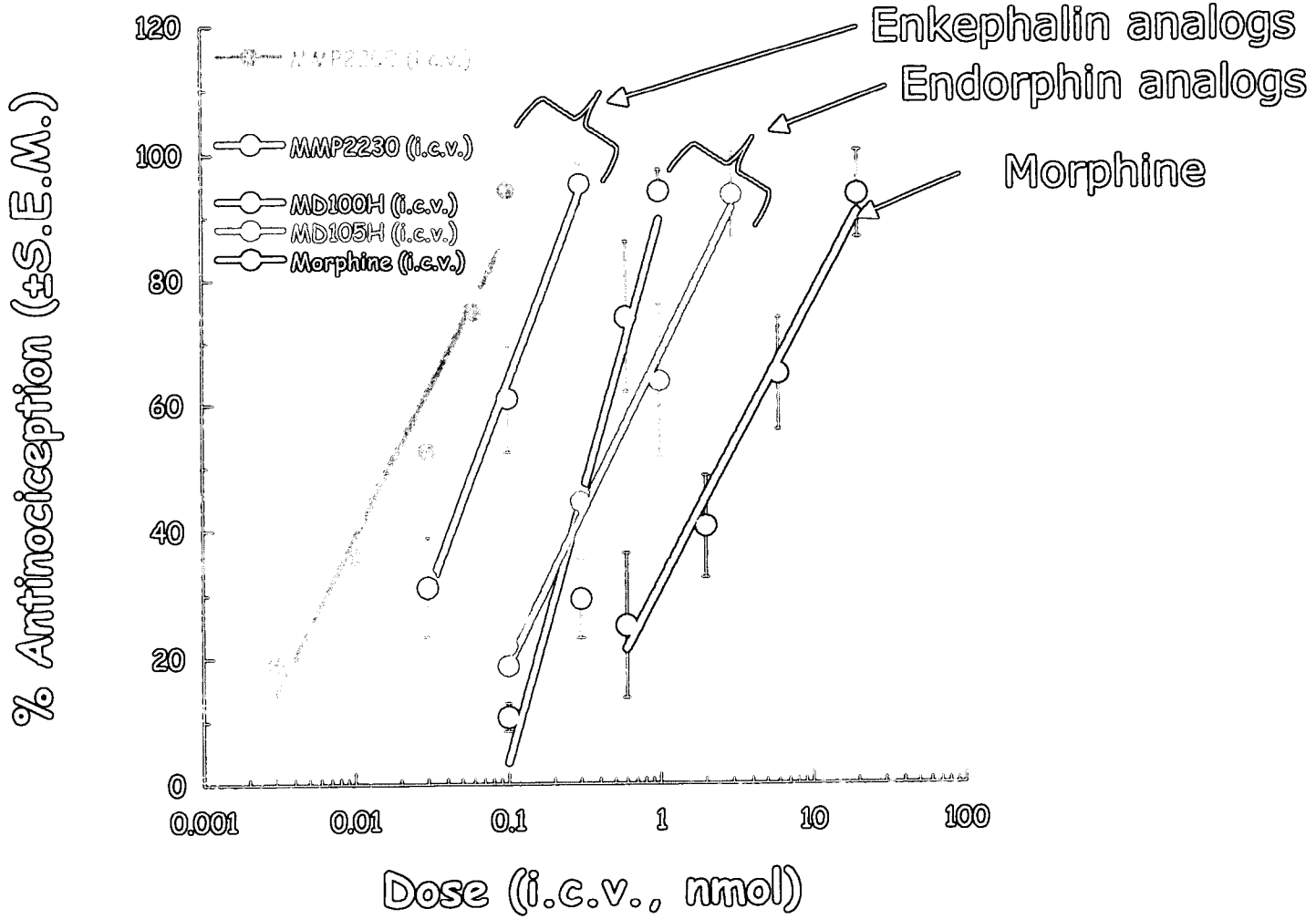
Endorphin/dynorphin analogs

H_2N-Y-(D)T-G-F-L-Linker-N-B-E-K-A-L-K-Ser(Glc)-L-NH_2

Linker	MVD (δ) IC_{50} nm	GPI (μ) IC_{50} nm	μ / δ selectivity
Pro	34.49	63.14	1.8
βAla	22.95	353.7	15.4
Gly-Gly	18.79	196.4	10.4
Morphine	258	54.7	0.212

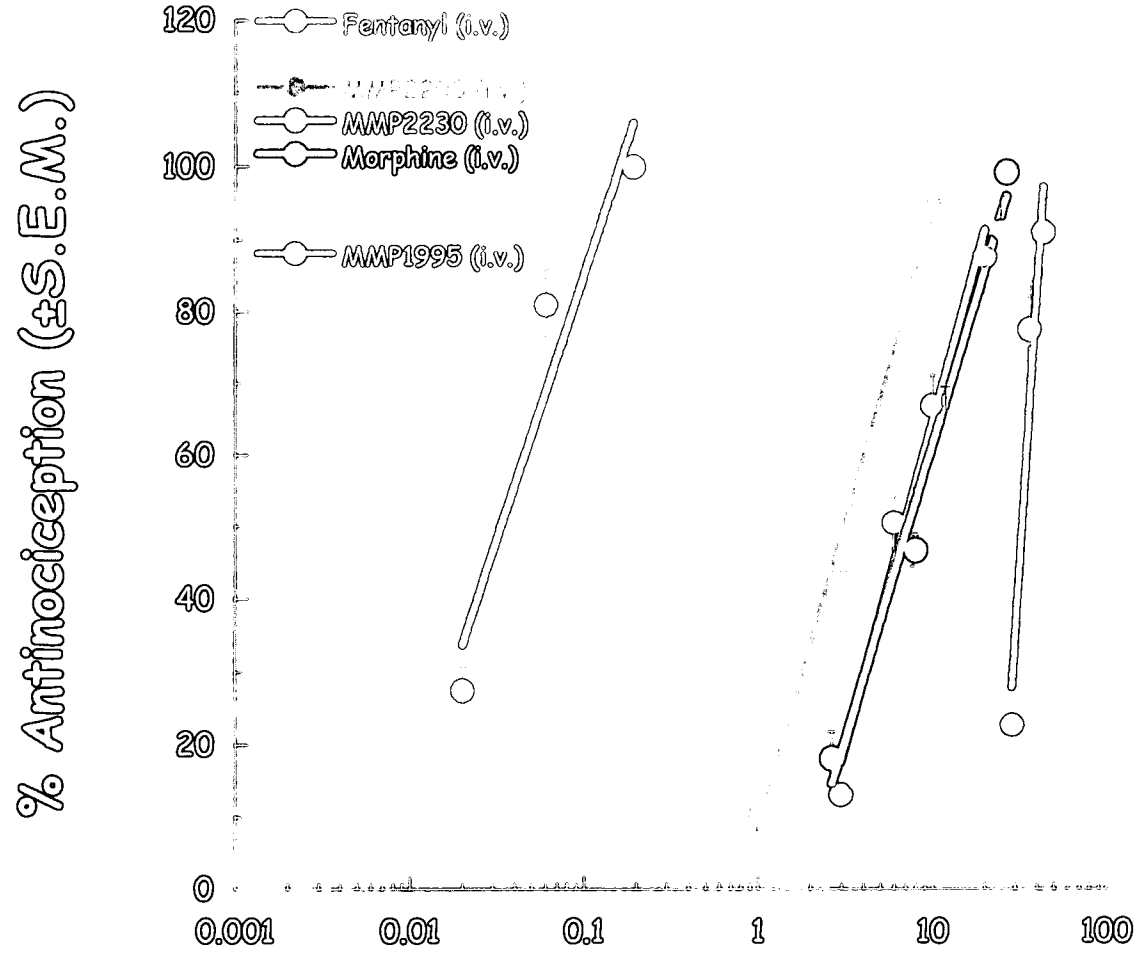
Peg Davis, Dept of Pharmacology, University of Arizona

In vivo pharmacology: Analgesics in mice i.c.v.



Ed Bilsky, University of New England, Maine

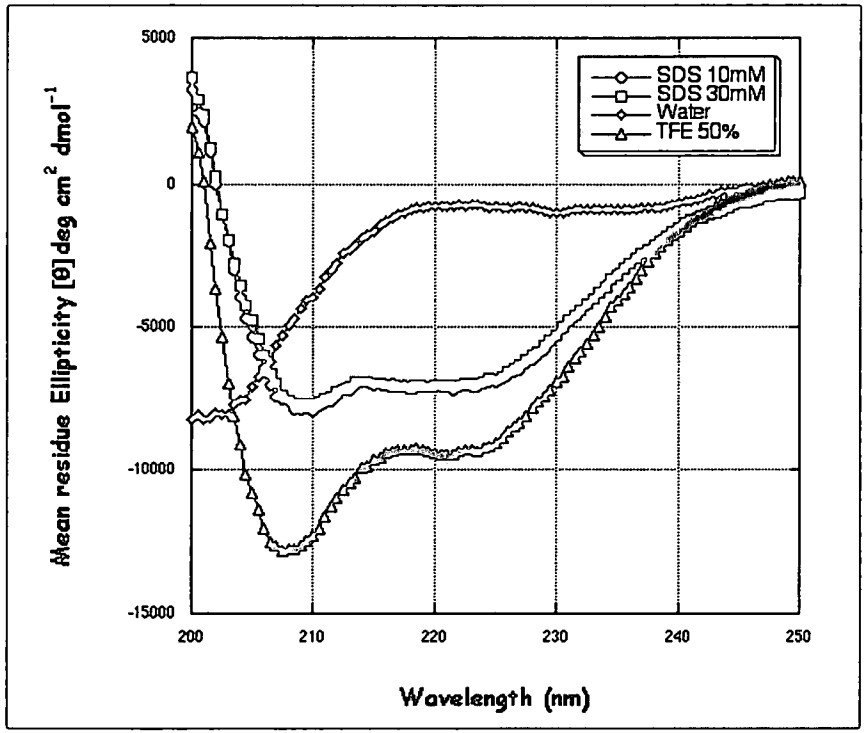
In vivo pharmacology: Analgesics in mice i.v.



Ed Bilsky, University of New England, Maine

Peptide conformation by Circular Dicroism

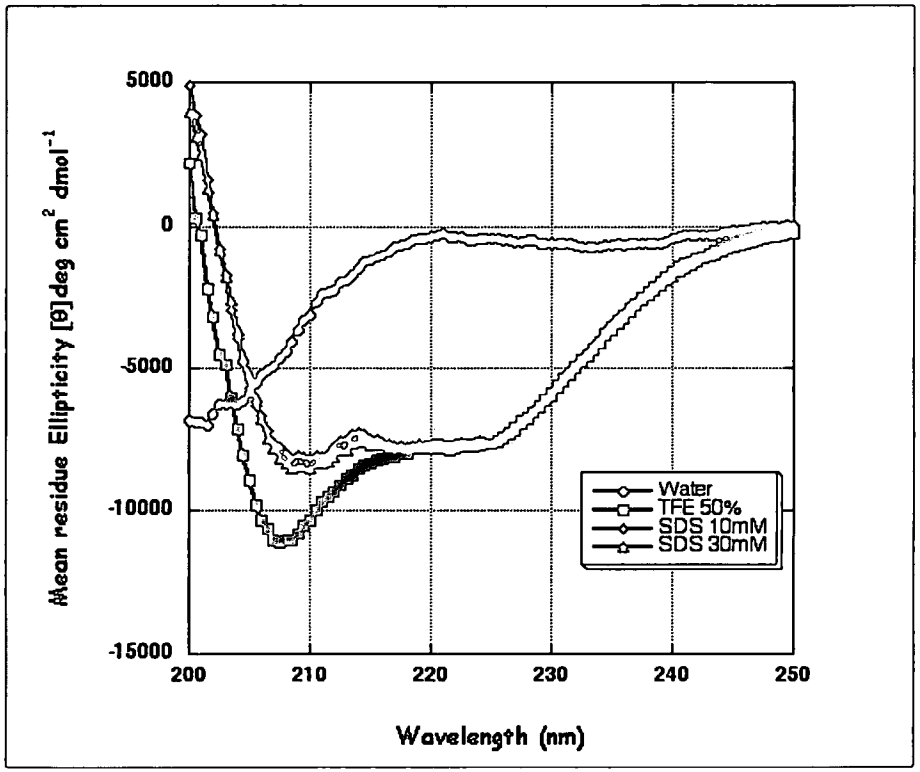
H_2N-Y-(D)T-G-F-L-Pro-N-L-B-E-K-A-L-K-Ser(Glc)-L-NH_2



- Peptide is random coil in water
- Adopts helical conformation in TFE and SDS micelle

Peptide conformation by Circular Dicroism

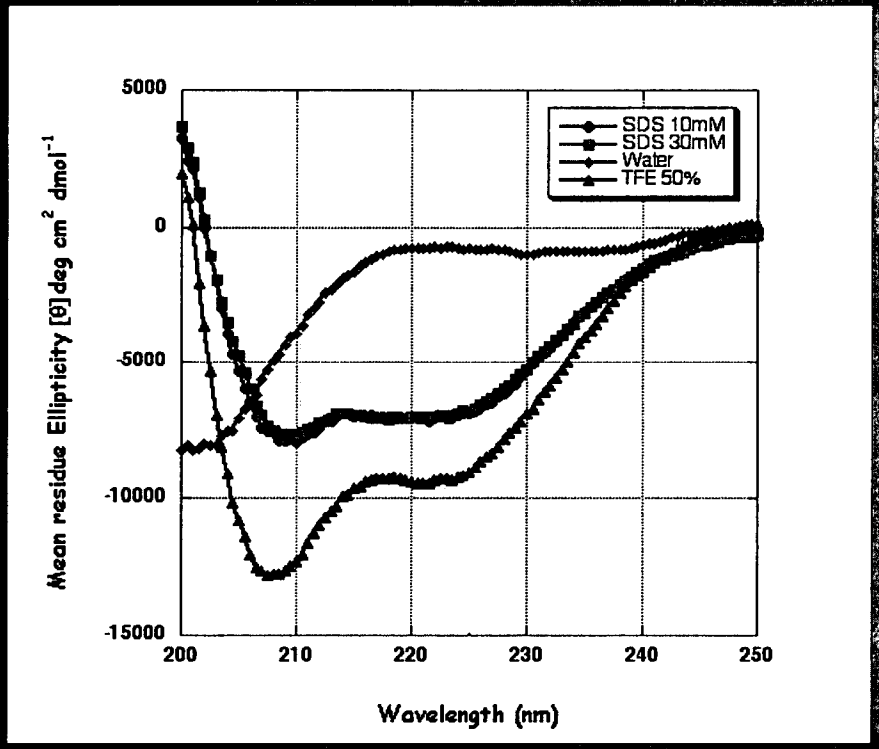
H_2N-Y-(D)T-G-F-L-Pro-N-L-B-E-K-A-L-K-Ser(Lac)-L-NH_2



- Peptide is random coil in water
- Adopts helical conformation in TFE and SDS micelle

Peptide conformation by Circular Dicroism

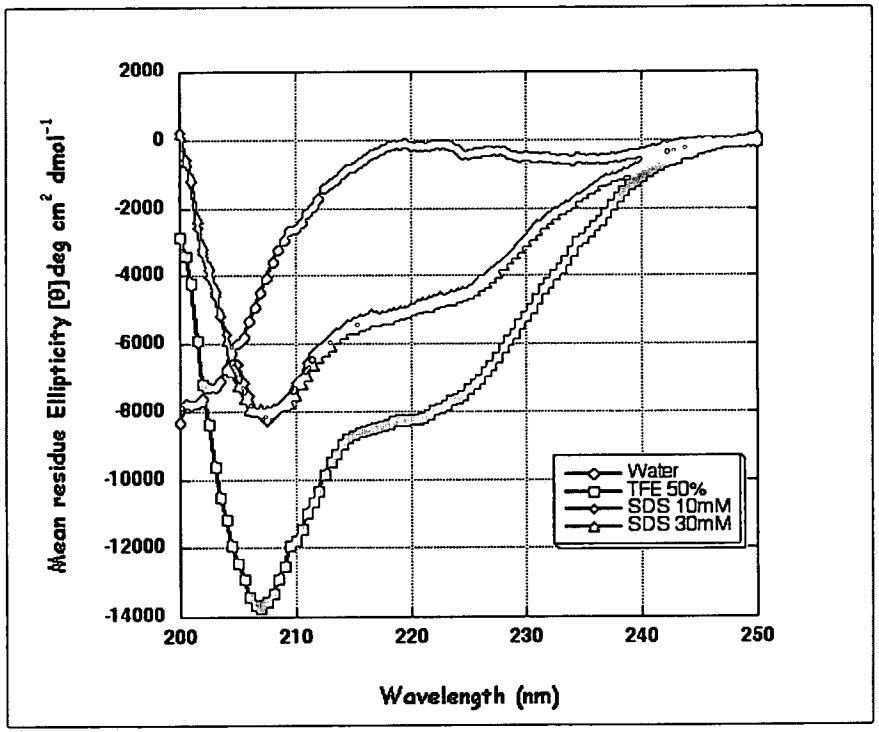
H2N-Y-(D)T-G-F-L-βAla-N-L-B-E-K-A-L-K-Ser(Glc)-L-NH2



- Peptide is random coil in water
- Adopts helical conformation in TFE and SDS micelle

Peptide conformation by Circular Dicroism

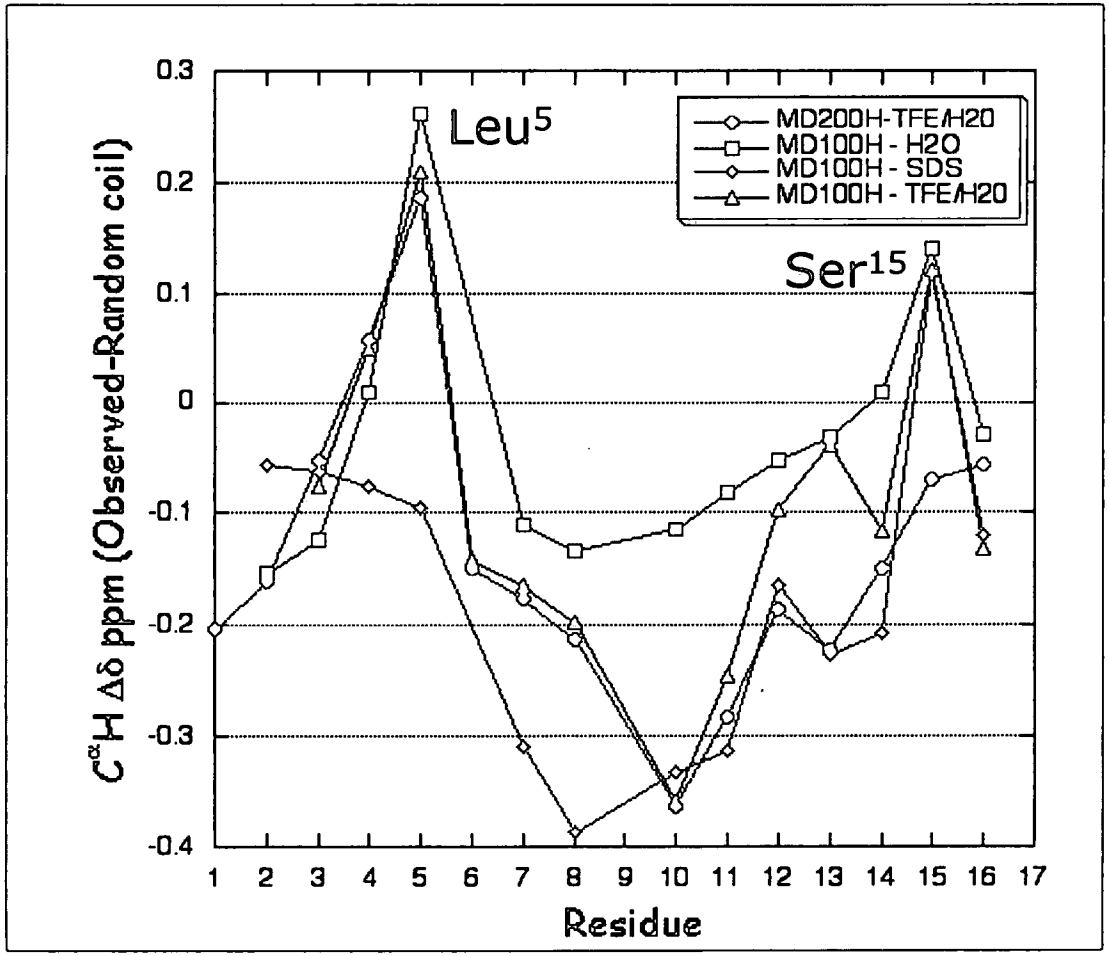
H2N-Y-(D)T-G-F-L-Gly-Gly-N-L-B-E-K-A-L-K-Ser(Glc)-L-NH2

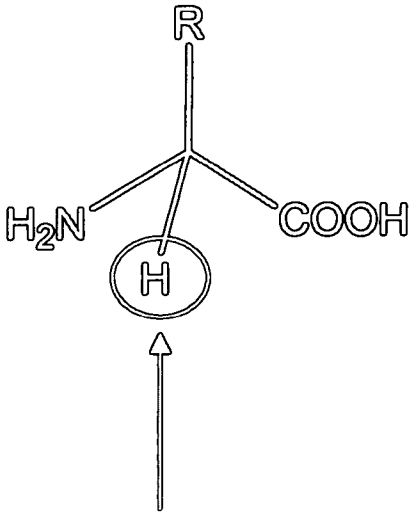


- Peptide is random coil in water
- Adopts helical conformation in TFE and SDS micelle

Peptide structure by ^{1}H-2D NMR: Chemical shift plot

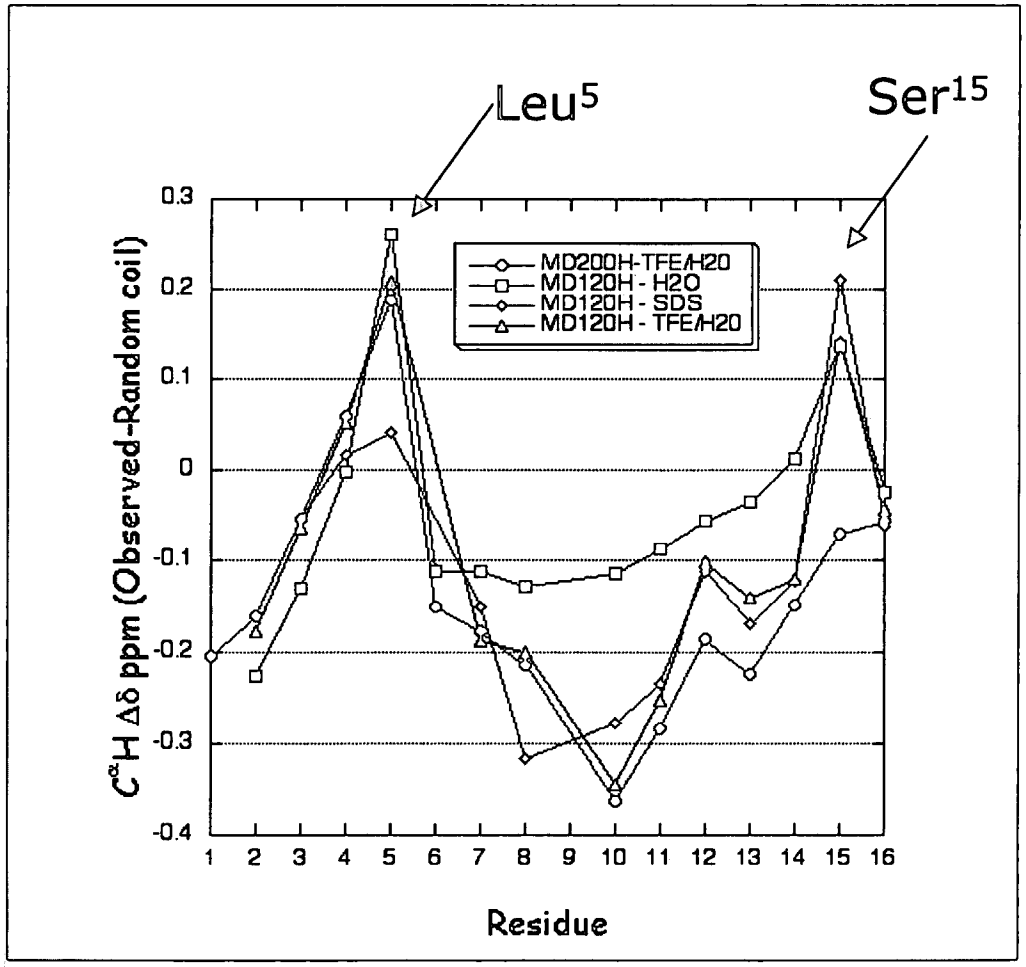
H_2N-Y-(D)T-G-F-L-Pro-N-L-B-E-K-A-L-K-Ser(Glc)-L-NH_2





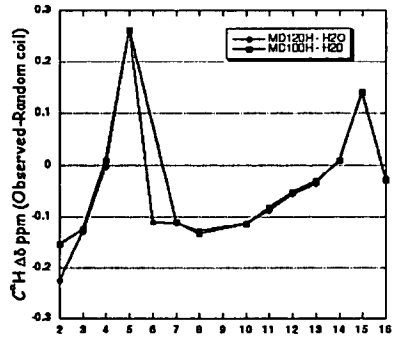
Peptide conformation by ^{1}H-2D NMR: Chemical shift plot

H_2N-Y-(D)T-G-F-L-<u>Pro</u>-N-L-B-E-K-A-L-K-<u>Ser(Lac)</u>-L-NH_2

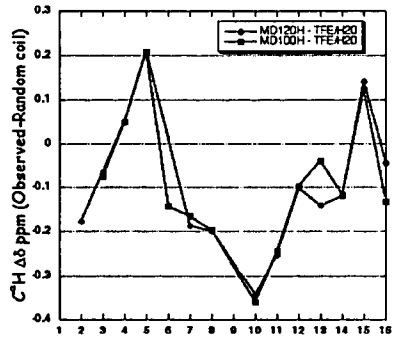


Effect of different sugars on peptide conformation

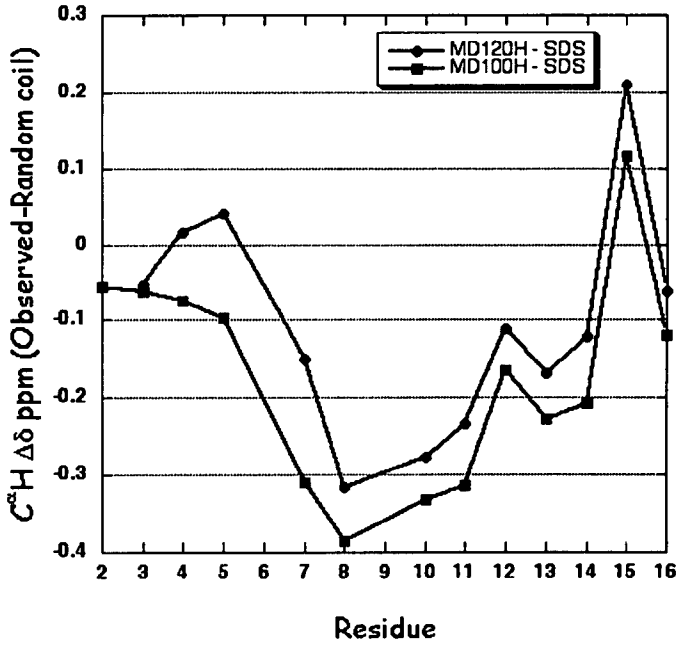
Water



TFE-Water mixture

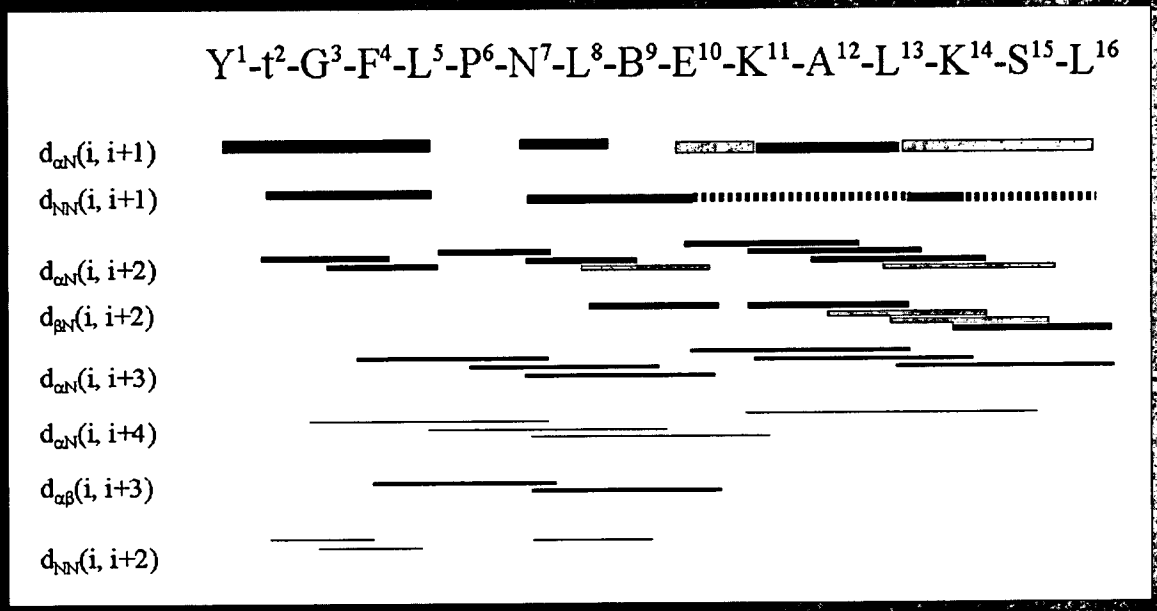


SDS micelle



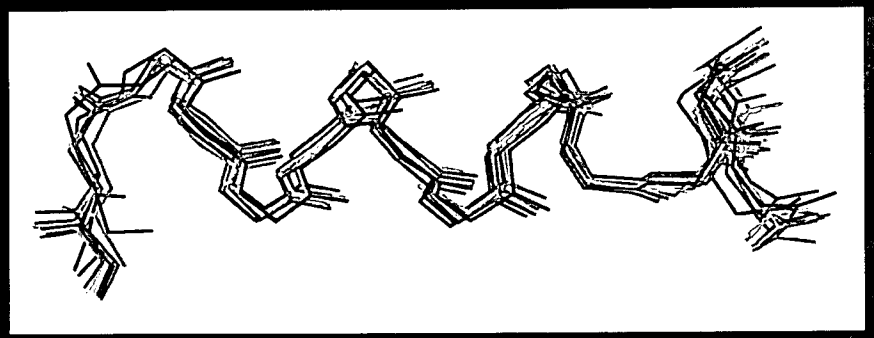
Peptide conformation in SDS micelle by ^{1}H-2D NMR

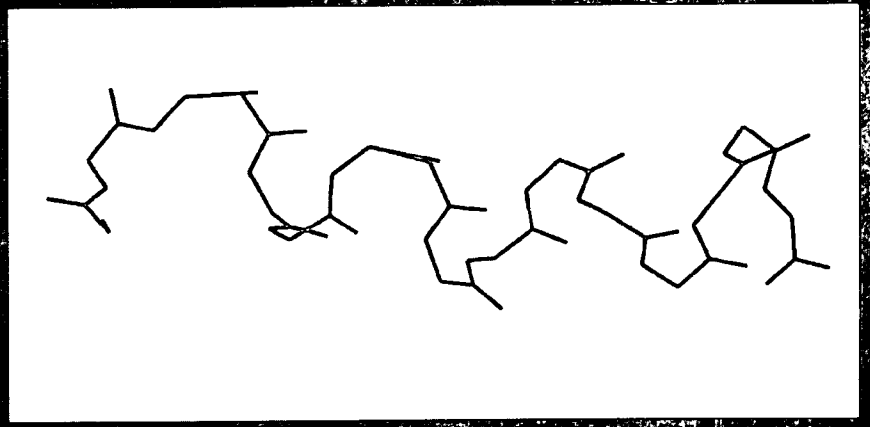
H_2N-Y-(D)T-G-F-L-Pro-N-L-B-E-K-A-L-K-Ser(Glc)-L-NH_2

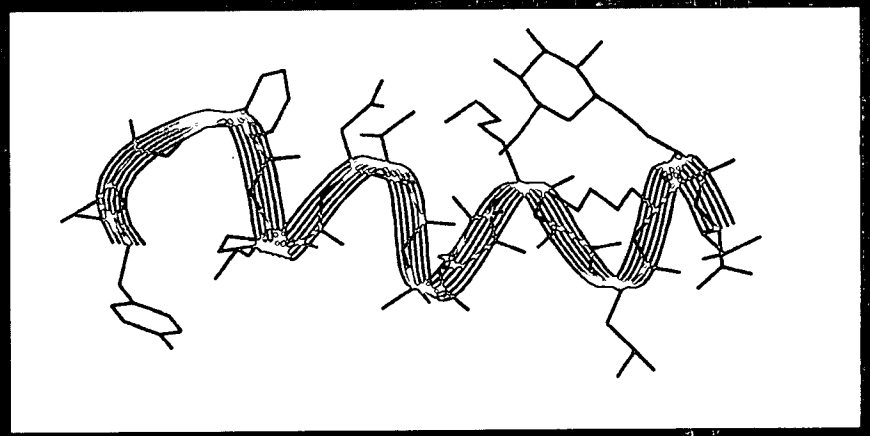


Peptide conformation in SDS micelle by ^{1}H-2D NMR

H_2N-Y-(D)T-G-F-L-Pro-N-L-B-E-K-A-L-K-Ser(Glc)-L-NH_2

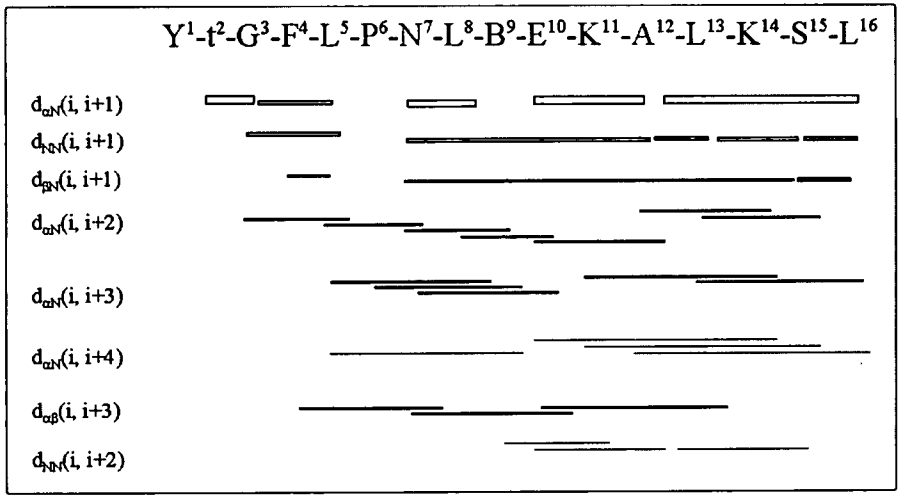


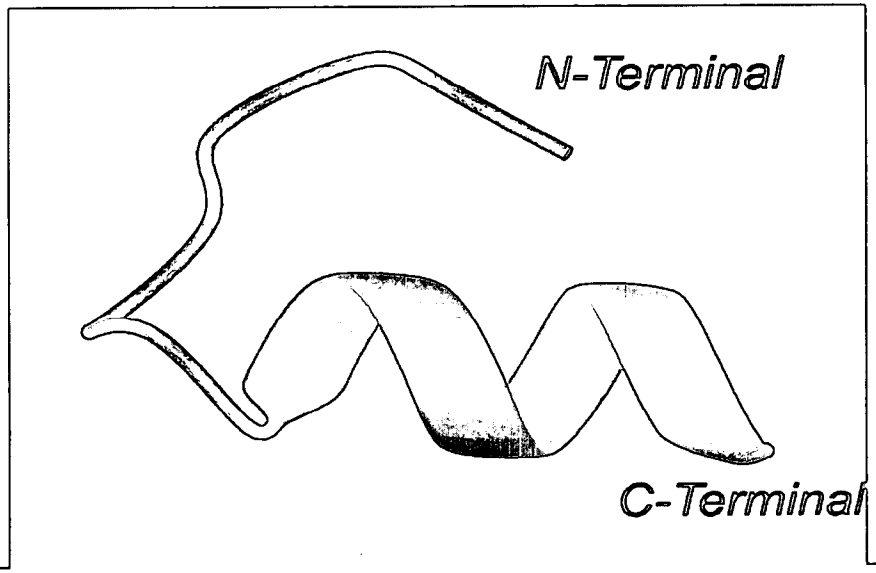




Peptide conformation in SDS micelle by ^{1}H-2D NMR

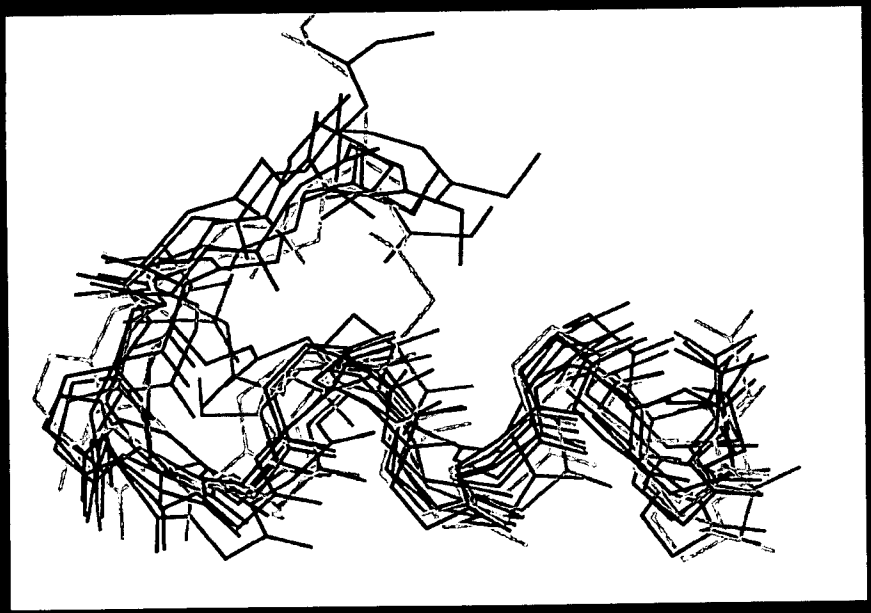
H_2N-Y-(D)T-G-F-L-Pro-N-L-B-E-K-A-L-K-Ser(Lac)-L-NH_2

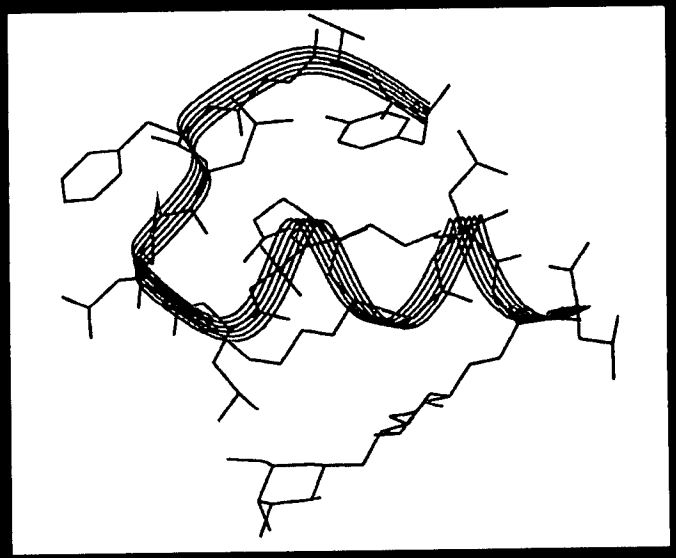


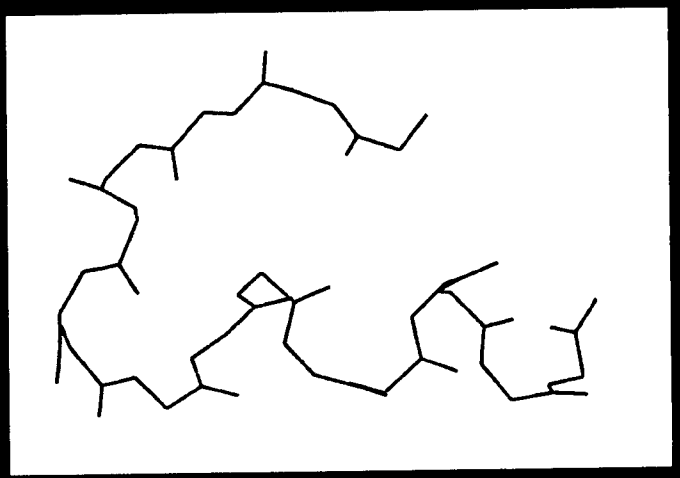


Peptide conformation in SDS micelle by ^{1}H-2D NMR

H_2N-Y-(D)T-G-F-L-<u>Pro</u>-N-L-B-E-K-A-L-K-<u>Ser(Lac)</u>-L-NH_2

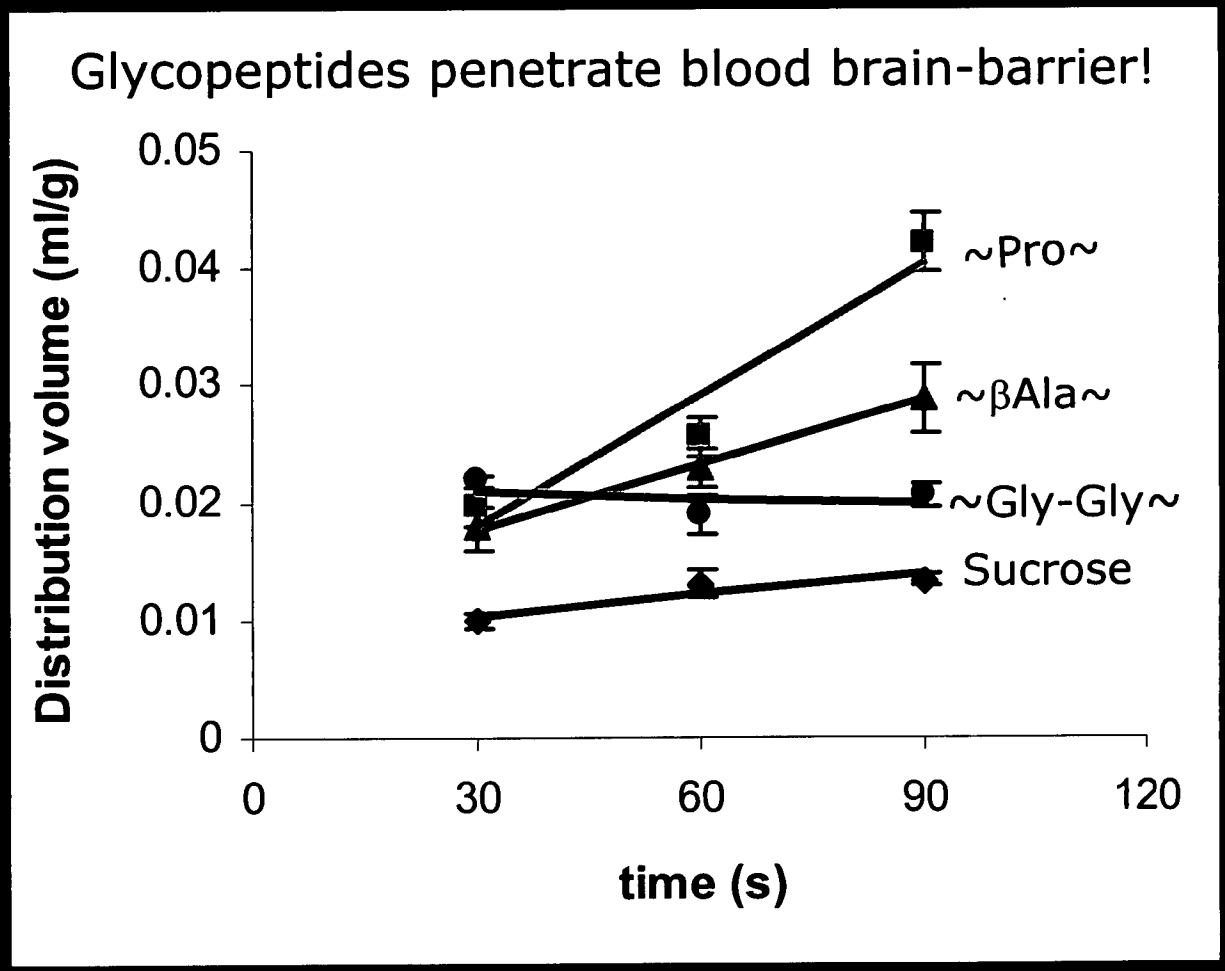






Mouse in situ perfusion studies

H_2N-Y-(D)T-G-F-L-Linker-N-L-B-E-K-A-L-K-Ser*-L-NH_2



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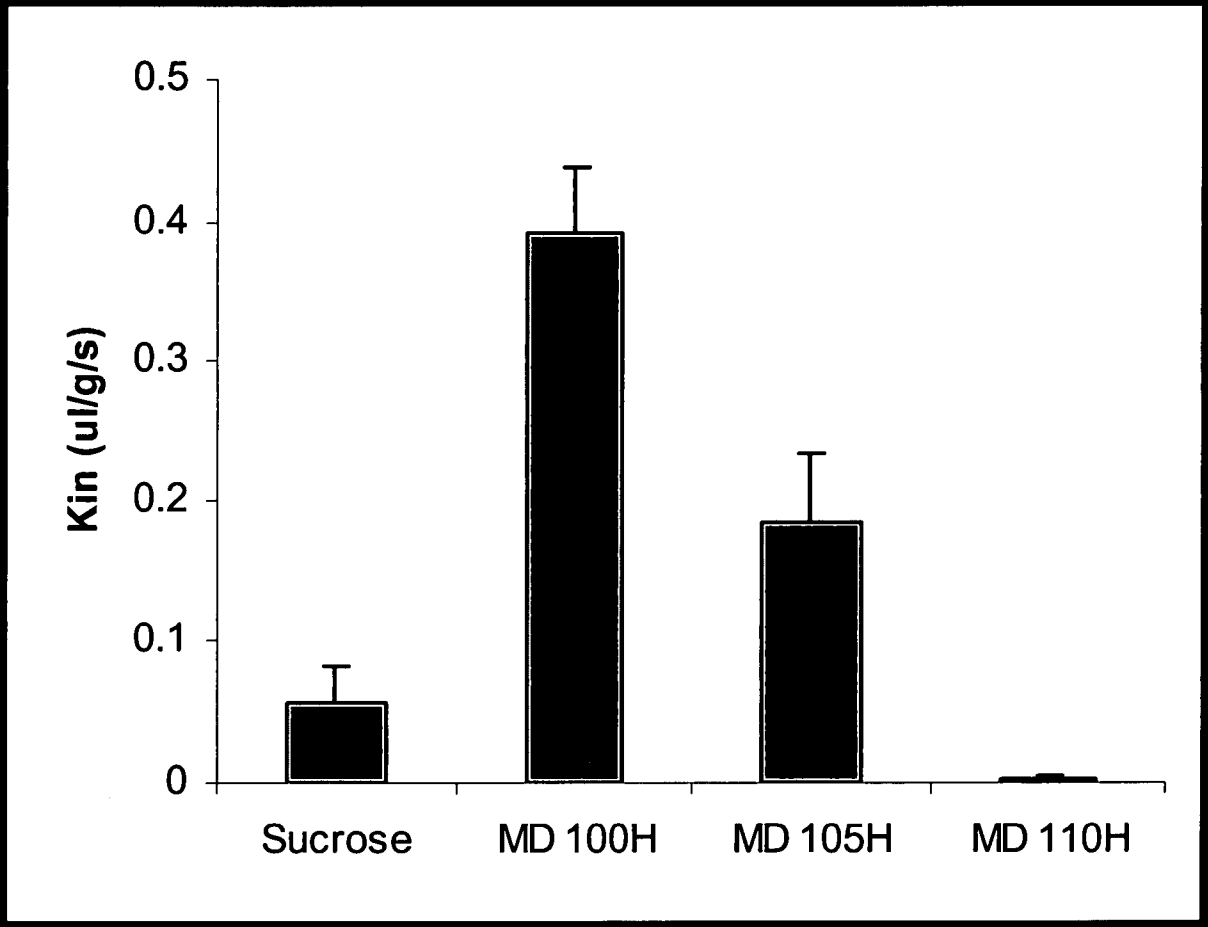
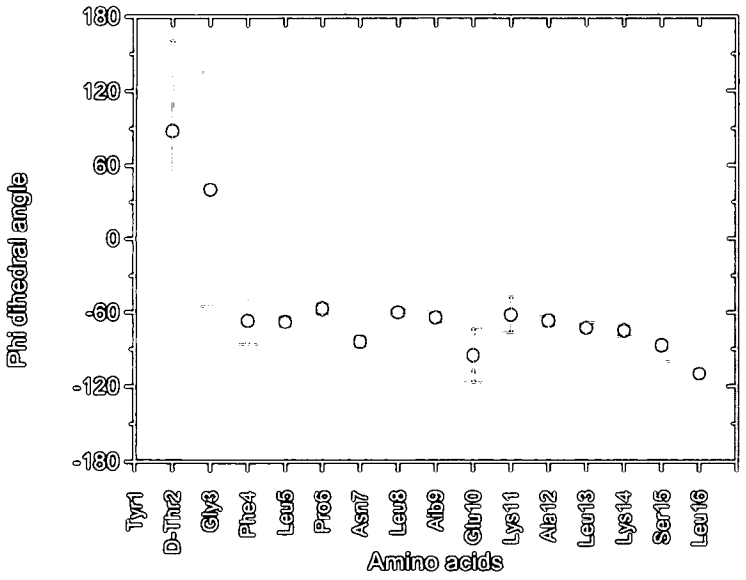
Kinetic values from in situ perfusion studies

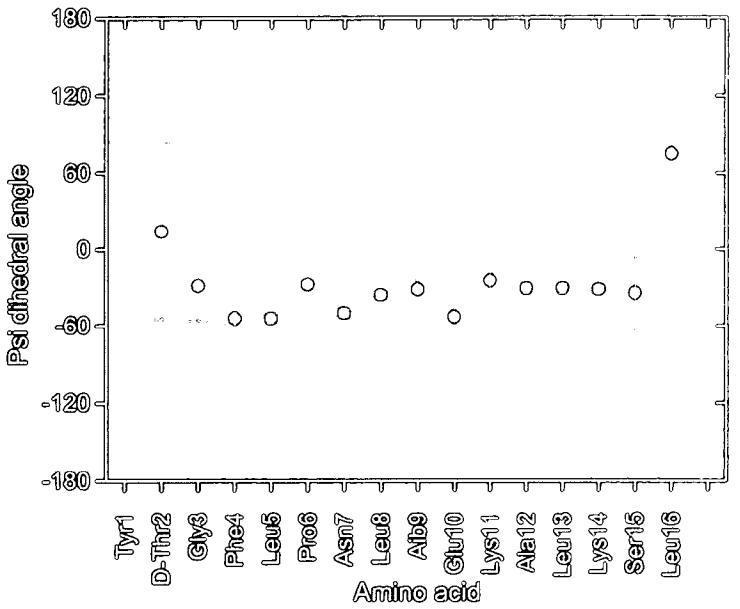


Conclusions

- Glycosylation promotes blood brain-barrier penetration irrespective of the length of the peptide
- Peptide with Proline linker is the best among endorphin analogs and is potential candidate for further development
- Sugar type perturbs the conformation of the peptide in amphipathic media

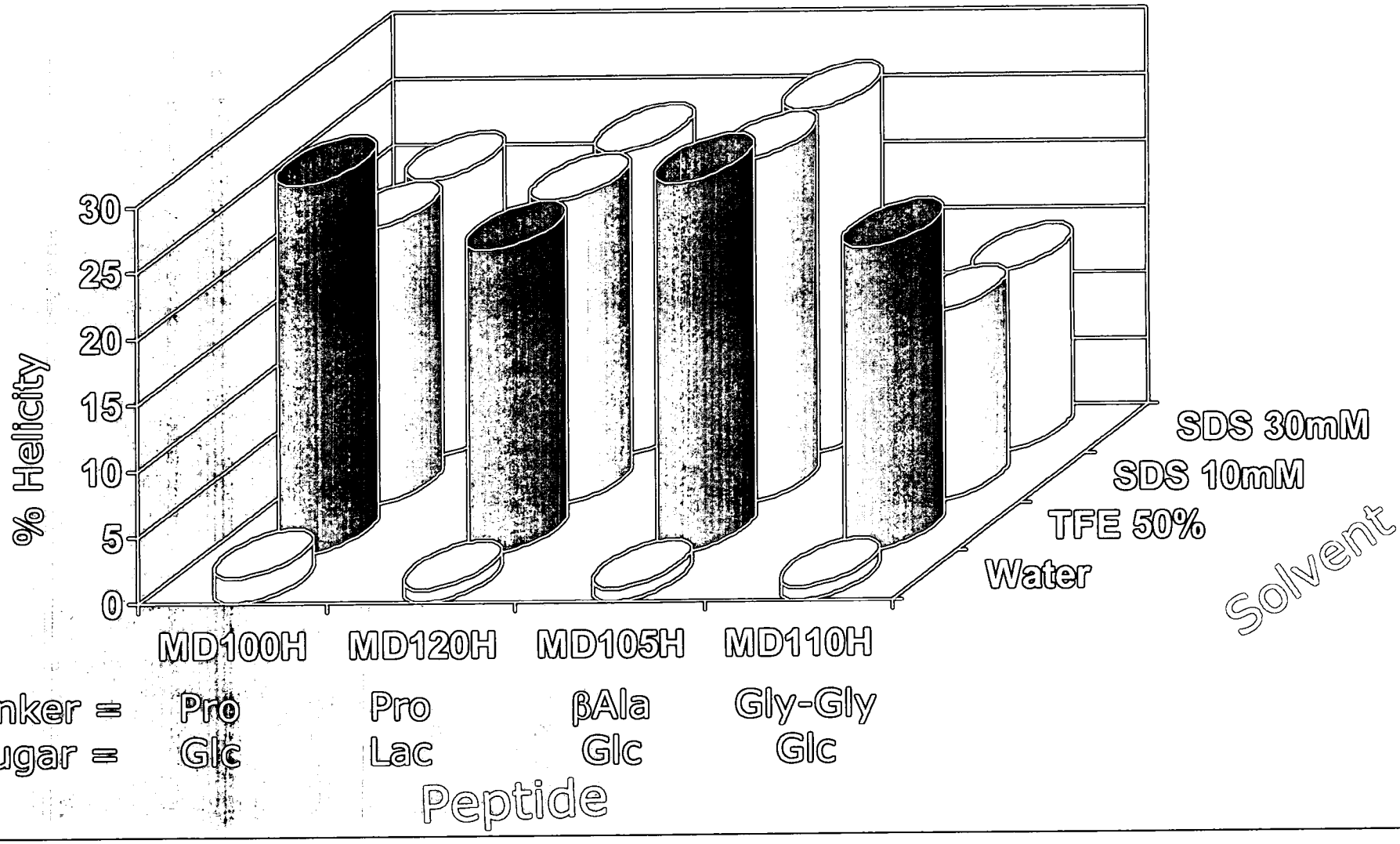
Dihedral angle distribution over 200ps MD analysis



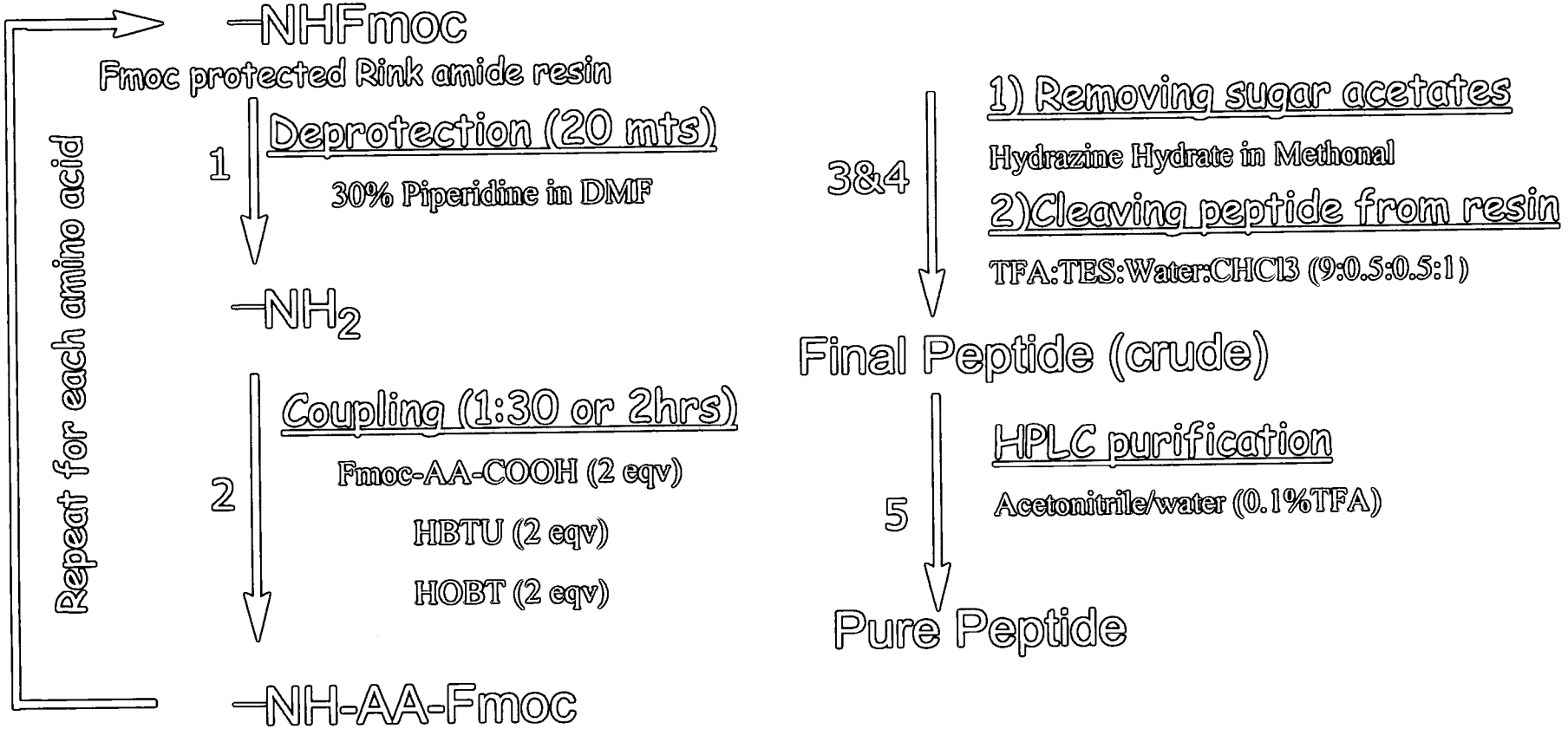


Peptide 100h in sds

Percentage helicity from circular dichroism

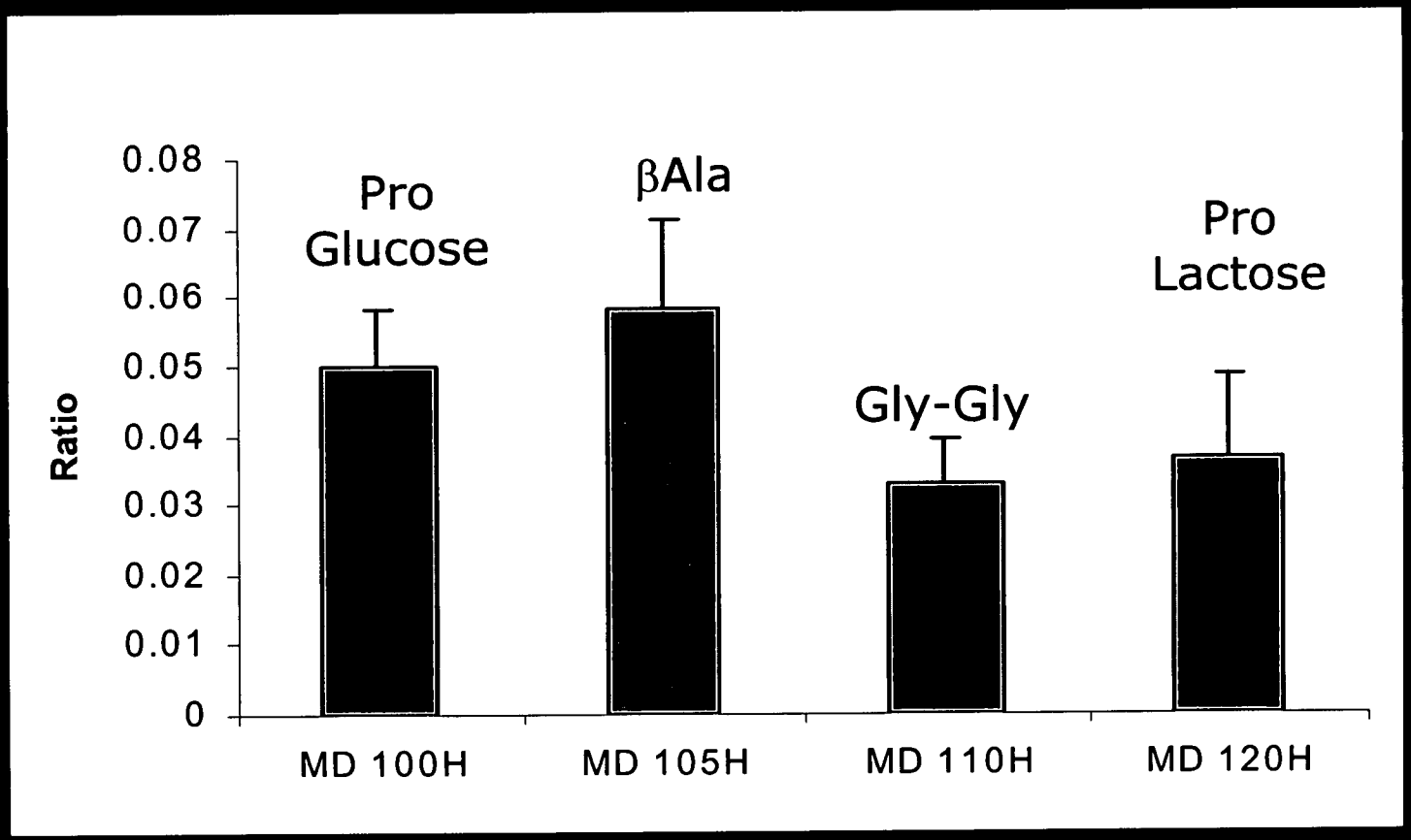


Peptide synthesis



Octanol:saline distribution studies

H_2N-Y-(D)T-G-F-L-Linker-N-L-B-E-K-A-L-K-Ser*-L-NH_2



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